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EVOLUTIONARY MATCHING OF PHYSIOLOGICAL CAPACITIES TO NATURAL LOADS. Jared Diamond. Physiology Department, UCLA Medical School, Los Angeles, CA 90024.

A major unsolved problem at the interface between physiology and evolutionary biology is: what sets the quantity of any physiological or anatomical component? For example, how are an enzyme’s activity, a tissue’s mass, a bone’s strength, or an ion channel’s membrane density determined? Much of modern biology is concerned with the proximate mechanisms setting these quantities, such as rates of protein synthesis and degradation. However, all of these quantities also pose rarely-discussed questions of ultimate causation: how did natural selection come to set those rates of protein synthesis and degradation, hence those enzyme activities or tissue masses, at their observed levels? For any physiological component one can calculate its safety factor as the ratio of its capacity to its maximum natural load. It turns out that measured safety factors mostly fall in the range 1.2 - 10. This observed variation in safety factors can be understood in terms of the varying costs and benefits of excess capacity. Such evolutionary considerations provide a quantitative framework for understanding physiological design.

NEURAL MODULATION OF MUSCLE PROPERTIES

2.1 DEVELOPMENTAL CHANGES IN PEPTIDE NEUROMODULATORS IN INSECT MOTORUNNERS: IMPLICATIONS FOR THE NEUROMUSCULAR SYSTEM. J. Witten. Dept Biol Sci, Univ Wisconsin, Lamphur Hall, PO Box 413, Milwaukee, WI 53201.

Nervous systems must be able to adapt to constantly changing environments to enhance animal survival. One way to generate neuronal and behavioral flexibility is to regulate neurotransmitter expression. Changes in neurotransmitter expression occur during development, growth and maturation, yet little is known about the physiological consequences of such alterations. My current research focus is to investigate the functional significance of developmentally regulated changes in neurotransmitter expression and its relationship to motor events underlying behavioral plasticity.

The moth, Manduca sexta, is a compelling model system for these studies since it permits analysis of the neural basis for behavioral plasticity at the molecular, cellular, organismal, and whole animal level. My research focuses on functions for developmental alterations in FMRFamide peptide (FaP) expression. Dramatic changes in the expression of a specific FaP occur in identified motoneurons during the life of the moth. Such changes are correlated with stage-specific alterations in body tone and movements. FaP is not detected during embryogenesis (Gruhl and Witten, 1994). It appears gradually during the larval stage and declines during metamorphosis, under the influence of the changing ecdysteroid titers. It is absent in the newly emerged adult, even though the motoneurons and muscles persist (Witten and Truman, 1990). By following the fate of one identified motoneuron and its target throughout the life of the moth, the functional consequences for changes in transmitter expression will be assessed.

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Do FMRFamide peptides influence embryonic muscle development in the tobacco hornworm, Manduca sexta?
Vol. 20: 86.

Witten, J.L. and Truman, J.W.
Stage-specific expression of FMRFamide-like immunoreactivity in motoneurons of the tobacco hornworm, Manduca sexta, is mediated by steroid hormones.
Vol: 16: 633

2.2 PEPTIDE AND AMINE MODULATION OF INSECT NEUROMUSCULAR TRANSMISSION. Michael E. Adams, Larisa D. Acevedo and David N. Mbungu. Deps. of Entomology & Neuroscience, University of California, Riverside, CA 92521.

Proctolin and octopamine potentiate synaptic responses at body wall neuromuscular junctions of the larval house fly, Musca domestica. Modulation of both calcium and potassium channels contributes to these effects. Application of either proctolin or octopamine results in enhanced twitch contractions associated with the appearance of calcium action potentials in muscle cells, effects that are mimicked by repetitive nerve stimulation. These effects are associated with increased currents through L type calcium channels in voltage-clamped muscle cell. Calcium channel modulation is mimicked by phorbol ester and blocked by H-7, suggesting that both peptide and amine actions are mediated postsynaptically by protein kinase C. Proctolin also produces a slow depolarization of the muscle resting potential resulting from decreased resting potassium channel conductance. Octopamine has additional pre- and postsynaptic actions. In addition to increasing the rate of spontaneous transmitter release, octopamine appears to elevate gap junctional conductance between adjacent muscle cells persisting for up to 1 hr after removal of the amine. The combined actions of the peptide and amine modulators at this synapse result in potentiation of the postsynaptic response and increased electrical coupling between adjacent muscle segments, thus facilitating muscle performance and coordination during locomotory behaviors.

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2.3 Neural modulation of muscle properties by RFamide peptides in the leech parallel the RFamide modulation of neural properties. R.L. Calabrese, Biol. Sc, Emory U, Atlanta, GA 30322.

Five neuropeptides terminating in the sequence RFamide have been isolated and sequenced from hemolymph extracts of the medicinal leech (Evans et al., 1991). These peptides have been localized to a number of motor neurons in the leech, including identified heart and longitudinal muscle motor neurons and also to specialized efferents that modulate the hearts. HA neurons (Kuhlman et al., 1985). The RFamide peptides induce tonic muscle contractions and, in the case of the hearts, myogenic contraction rhythms when superfused "in situ". Voltage clamp studies reveal that in heart muscle, RFamide peptides modulated heart rate activity by activating a voltage-dependent Ca2+ current, modulating outward currents (Thompson and Calabrese, 1992). RFamide peptides have also been immunocytochemically localized to interneurons that modulate the period of the centrally generated heartbeat rhythm. Biophysical analyses indicate that RFamide peptides modulate outward currents and a Na+ current in the interneurons of the heartbeat central pattern generator.


Molluscan neuromuscular junctions very commonly incorporate local modulatory mechanisms that shape contractions to suit behavioral demands. The physiological roles and cellular mechanisms of such modulation are best understood in the ARC-muscle system of Aplysia. The ARC (accessory radula closer) muscle participates in feeding behavior. Its two motorneurons B15 and B16 release ACh to contract the muscle, but also numerous peptide cotransmitters of several families - SCPs, myomodulins, and buccalins - that variously potentiate, depress, and accelerate the rate of the ACh-induced contractions. In addition to this "extrinsic" modulation, serotonin from "extrinsic" modulatory MCC neurons has similar effects. The motorneurons release ACh and/or the peptides with different stimulation patterns recorded in freely feeding animals. Potentiation of the contractions strengthens the drive e.g. during food-induced arousal, and acceleration of their relaxation rate may help maintain synchronization of contractions of different muscles required for functional behavior. The modulators act at presynaptic autoreceptors to alter ACh release, as well as directly on the postsynaptic ARC muscle itself. Studies of the electrophysiology and contractions of single ARC muscle fibers have identified CAMP-dependent enhancement of Ca2+ current as the major mechanism of the potentiation of contractions, and activation of K current of the depression. Currently, phosphorylation of the giant muscle protein twitchin appears the best candidate mechanism for the acceleration of the relaxation rate. Finally, cDNAs encoding multiple myomodulins and buccalins have been cloned. Many of these findings in the experimentally advantageous ARC-muscle system are likely to reflect general features of neuromuscular modulation.

2.5 NEURAL MODULATION OF MUSCLE PROPERTIES: SHARING OF NEURAL AND NON-NEURAL CONTROL OF MUSCLE PROPERTIES IN MAMMALIAN SYSTEMS. V. Reggie Edgerton, Roland E. Roy and John A. Hodgson. Dept. of Physiological Science and Brain Research Institute, Los Angeles, CA 90024.

An influence of the nervous system on skeletal muscle properties in mammalian systems has been evident for years, however the level of control has not been well-defined. The experiments of Boller and colleagues in the late '50s and early '60s demonstrated that slow skeletal muscles become faster and fast muscles became slower when the muscle was denervated and subsequently reinnervated by a nerve that originally innervated a fast or slow muscle, respectively (1). Through the '60s, the prevailing concept was that the nervous system had complete control of all muscle fibers and, further, that this control was exerted by modulation of the number and, to some extent, the frequency of activation of the muscle fiber. More recently, it has become clear that the neural control of muscle fiber properties is not complete (2,3). The relative level of control of specific physiological, morphological, and molecular characteristics from neural and non-neural sources will be discussed.

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PLASTICITY IN INSECT NEUROMUSCULAR SYSTEMS: DEVELOPMENTAL AND EVOLUTIONARY PERSPECTIVES. Arbas, EA. ARL Div. of Neurobiology & Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85721.

The degree of atrophy exhibited in neumomuscular systems following trauma varies greatly over the lifespan of an animal, with the immature system typically showing greater regression than the terminally developed adult system and, often, greater specificity during recovery. We have studied the consequences, on an insect neuromuscular system, of nerve trauma that accompanies shedding of the hindlimb by autotomy. In certain grasshoppers, such as the flightless \textit{B. psolus}, damage occurring to the leg nerve (N5) during autotomy, transneuronally induces atrophy and muscular regression. When undamaged, fully innervated muscles intrinsic to the thorax. While the experimental muscles are reduced to <15% of control mass in \textit{B. psolus}, similar treatment of related taxa, \textit{S. americana} and \textit{M. americanus}, induces a less severe atrophy (to 70-80% of control in the former, 60-90% in the latter) in a small subset of these muscles. These differences may result from evolutionary changes in ontogenetic trajectories in the species studied. Sensitivity to hindlimb autotomy is accentuated in early larval stages of \textit{S. americana} and \textit{M. americanus} to an extent nearly equivalent to that in \textit{B. psolus}, and dimished with maturation to adulthood (i.e. there is a developmental sensitive period for this effect), a situation comparable to many vertebrate species. Various behavioral and morphological changes of \textit{B. psolus} suggest that it is permanently juvenilized (paedomorphic) relative to its taxonomic cousins. It's marked sensitivity to hindlimb autotomy, as revealed by muscle atrophy and alteration of a suite of cellular and synaptic properties, persists throughout life. While most workers control for developmental stage in studies of neuromuscular plasticity, our studies emphasize the need also to assess evolutionary distortions of ontogenetic trajectory when making comparisons across taxa. [Supported by NSF IBN-9210394].

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BIOMEDICAL APPLICATION OF MARINE MAMMAL PHYSIOLOGY: ADAPTATION TO AN AQUATIC WORLD

3.1
FASTING IN SEALS: CONTROL OF METABOLIC DEMAND
Daniel P. Costa, Jeanne Williams & Daniel Crocker
Department of Biology, University of California, Santa Cruz, CA. 95064.
Many marine mammals undergo prolonged periods of complete abstinence from food or water during the reproductive season, which may be the most energy intensive stage in an animals life cycle. For example, male northern elephant seals, \textit{Mirounga angustirostris}, fight to maintain dominance over females for periods of up to 3 months, and females nurse their pups over a 28 day period all while abstaining from ingestion of food or water. During this time the pups grow from 40 kg at birth to 150 kg at weaning. Once weaned they then fast for 2-3 months prior to departure from the breeding beach and presumably begin feeding at sea. Our research group has examined the water balance, energy metabolism, protein turnover, glucose turnover, and blood chemistry parameters of elephant seal pups and adults. This work suggests that these animals regulate their metabolism differently than dogs, rats and humans. Blood glucose and triglyceride levels are elevated during fasting; they are intolerant to glucose, due to a lack of insulin secretion; free fatty acids levels are extraordinarily high, while keto-acid levels remain low throughout the fast. Accommodation to a high fat diet may allow us to use the elephant seal as a novel model to study aspects of lipid metabolism and regulation that would be difficult to study due to their secondary role in a more "typical" mammal.

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Changes in resting metabolic rate during long-term fasting in northern elephant seal pups (\textit{Mirounga angustirostris}). 1991.

Relationship between plasma ketones and fasting duration in neonatal elephant seals.

3.2
BIOMEDICAL LESSONS FROM THE STUDY OF APNEA IN MARINE MAMMALS. W.K. Milsum and D.R. Jones
Department of Zoology, Univ. of British Columbia, Vancouver, B.C., V6T 2A9, Canada.
While prolonged apnea during diving is a well studied phenomenon in marine mammals, many of these mammals may also exhibit prolonged apnea during sleep, even on land. Studies of the underlying control of these apneas and their associated cardiovascular changes yield a number of important insights. 1) The cardiovascular changes associated with diving apnea, which include bradycardia and changes in blood flow distribution, have been well studied and their adaptive value to young infants in cases of near drowning in cold water are well established (1). 2) Diving apneas can be initiated by several reflex mechanisms. It's significance as a defensive mechanism is obvious but the strength of the reflex is often underplayed. If expressed as strongly in hypoxia tolerant infants, it could contribute to the sudden infant death syndrome (2). 3) Sleep apnea in seals is a central apnea that might result from a reduction in metabolic rate to the point where continuous ventilation is no longer necessary. This may be similar to some forms of central sleep apneas in man, particularly in obese humans displaying hypometabolic states. Although most sleep apneas in these individuals are obstructive, many are secondary to an initial central apnea and may be a pathologic consequence of these normal events. 4) Breathing never occurs during rapid eye movement sleep in northern elephant seals, even in cases of elevated respiratory drive, suggesting that the atonia of REM sleep may extend to the diaphragm in some instances (3).
3.4

BIOCHEMICAL IMPLICATIONS OF PRESSURE DIVING.

M. Castellini, Institute of Marine Science, University of Alaska, Fairbanks, AK 99775.

Many marine mammals are capable of diving to extreme depths on a routine basis (1). Elephant seals are known to reach almost 1800 m on some dives (2) and sperm whales can exceed 2000 m. In fact, marine mammals approach depths that are known to alter biochemical reactions in other marine species (3) and certainly exceed the pressure tolerance of human divers. In this project, we have examined how pressure impacts the metabolism of living red blood cells (RBC). In all species examined, including humans, the distinction is clear: incubation at 2000 psi (about 1400 m) significantly depresses the metabolism of terrestrial RBCs and either has no impact or enhances the metabolic rate of marine mammal RBC. RBC metabolism is defined by the rate of lactate production. Yet, studies of both tissue (cardiac) and RBC lactate dehydrogenase, while showing some alterations under pressure, cannot account for the difference in metabolic rate. This suggests that there may be differences in membrane properties or in other glycolytic enzymes. These avenues are being explored. In any case, there is clearly a difference in the biochemistry of diving species relative to pressure adaptation that does not exist in terrestrial mammals, including man.

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3.5

ASPHTA, ISCHEMIA AND OXYGEN MALAULUS: R. Elsner, S. Cyanar and O.D. Saugstad, Institute of Marine Science, University of Alaska, Fairbanks, AK 99775-1080 and Institute for Pediatric Research, National Hospital, Oslo, Norway.

One of the primary adaptations of seals for long divingREG is the selective distribution of blood flow favoring organs requiring an uninterrupted supply. Other regions are exposed to prolonged and intense vasocconstriction. Such ischemia would be expected to produce cell damage if blood flow were not resored. Reperfusion, while obviously essential, is also a source of oxygen-derived tissue radicals. This condition results from production of hypoxanthine from ATP degradation and conversion of xanthine dehydrogenase to xanthine oxidase along with re-introduction of molecular oxygen. These events lead to cell membrane and protein damage (1). Naive organs are notably resistant to effects of long ischemia. Isolated harbor seal kidneys tolerated 60 min of warm ischemia that severely damaged similarly-treated dog kidneys (2). Coronary blood flow ceased periodically during experimental dives (3). Mechanisms supporting ischemic tolerance are poorly understood. Hypoxanthine is produced by ischemic seal tissues, and its harmless disposition is suggested. (Supported in part by The American Heart Association. Alaska Affiliate; Alaska College Sea Grant Program and North Slope Borough Division of Wildlife Management.)

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3.6

MARINE MAMMAL ATHLETES: MODELS FOR FUNCTIONAL DIVERSITY IN MAMMALIAN LOCOMOTOR SYSTEMS. Terri M. Williams and Randall W. Davis. "Dept. of Biology, Univ. of CA, Santa Cruz CA 95064; Texas A&M Lufkin, TX 77553.

Secondarily aquatic mammals have developed a wide variety of metabolic and skeletal muscle adaptations in response to the conflicting physiological demands of exercise and diving. During a dive, the pathway for oxygen is interrupted at the level of the gills. The aquatic mammal must then use its own oxygen stores. The diving mammal has a higher affinity for oxygen than the terrestrial mammal, average blood flow to the skeletal muscles of marine mammals must be reduced. The diving mammal also has a higher muscle oxygen concentration, which ranges from 3 to 10 mg ml⁻¹ for terrestrial mammals. Therefore, the mammal can use its own oxygen stores. Thus, the paradoxical decrease in muscle blood flow is required when the metabolic demands of the skeletal muscle are increased during aerobic dives. These results on marine mammals demonstrate the plasticity of the circulatory and skeletal muscle systems when oxygen delivery is limited.

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RW Davis, MA Castellini, TM Williams, and GL Kooyman Fuel homeostasis in the harbor seal during submerged swimming. Journal of Comparative Physiology 160:627-635, 1991


REPLICA, ISCHEMIA AND OXYGEN MALAULUS: R. Elsner, S. Cyanar and O.D. Saugstad, Institute of Marine Science, University of Alaska, Fairbanks, AK 99775-1080 and Institute for Pediatric Research, National Hospital, Oslo, Norway.

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3.6

MARINE MAMMAL ATHLETES: MODELS FOR FUNCTIONAL DIVERSITY IN MAMMALIAN LOCOMOTOR SYSTEMS. Terri M. Williams and Randall W. Davis. "Dept. of Biology, Univ. of CA, Santa Cruz CA 95064; Texas A&M Lufkin, TX 77553.

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RW Davis, MA Castellini, TM Williams, and GL Kooyman Fuel homeostasis in the harbor seal during submerged swimming. Journal of Comparative Physiology 160:627-635, 1991

3.7  
BLOOD RHEOLOGY IN NEWBORN AND ADULT SEALS: PHYSIOLOGIC ADAPTATIONS OR RHEOLOGIC ABNORMALITIES? H. J. Meiselman, M. A. Castellini and R. Elsner. Dept. Physiology and Biophysics, USC School of Medicine, Los Angeles, CA 90033 and Institute for Marine Science, University of Alaska, Fairbanks, AK 99775

Seals place extreme demands on circulatory blood flow during prolonged dives, yet hemorheological information for these marine mammals is limited. We thus investigated several rheologic indices in elephant seals (ES, M. angustirostris), ringed seals (RS, P. hispida) and Weddell seals (WS, L. weddelli). Salient results included: 1) elevated hematocrit (ES=62, RS=51, WS=64%); 2) large MCV (ES=179, RS=122, WS=153 fl); 3) species-specific fibrinogen levels (ES=1.6, RS=1.7, WS=6.6 g/l). RBC aggregation was also species-specific: 1) extent of aggregation (ES=24, RS=0, WS=32, human=17); 2) aggregate strength (ES=105, RS=3, WS=220; human=61). Blood from newborn WS (24-36 hours old) exhibited very low RBC aggregation which increased toward adult WS values at 6-7 days post-partum. Blood viscosity data (40% RBC in plasma) indicated variations between species: WS blood was markedly non-Newtonian with elevated low shear viscosity, whereas RS blood exhibited much lower, nearly Newtonian viscosity. ES blood was intermediate in flow behavior. These results indicate marked rheologic "abnormalities" for seal blood, but are not associated with pathophysiologic findings, they suggest adaptive mechanisms and the value of aquatic mammals as model systems for circulatory studies.

EVOLUTION OF ENDOOTHERMIC METABOLISM

4.1  
ACTIVITY AND THE EVOLUTION OF ENDOtherMY IN MAMMALS AND BIRDS  
J.A. Ruben, Dept. of Zoology, Oregon State Univ.

Chronic, endothermically-based maintenance of high and stable body temperature (endothermic homeothermy) distinguishes mammals and birds from other Metazoa. Selective factors associated with the origin of elevated metabolic rates in these taxa have historically been the subject of considerable debate. Conventionally, selection for enhanced thermoregulatory capacity in mammalian and avian ancestors has been assumed to have been associated with the origin of amniote endothermy. In contrast, the aerobic capacity model hypothesizes that endothermic homeothermy was merely a fortuitous outcome of earlier selection for expanded aerobic capacity, associated with enhanced stamina and routine levels of activity. Reevaluation of recent physiological data, as well as new fossil evidence, supports the aerobic capacity model. Moreover, in the therapsid-mammal lineage, endothermy may have been achieved much earlier then the origin of Mammalia per se: in contrast, early birds and their immediate ancestors, dromaeosaurid dinosaurs, seem not to have attained endothermic metabolic status.

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4.2  
DIGESTIVE MECHANISMS AND CONSTRAINTS FOR FUELING THE METABOLISM OF BIRDS. William H. Karasov, Univ. of Wisconsin, Madison, WI 53706

Birds exhibit high rates of metabolism and hence higher feeding rates than any other vertebrate group. In addition, data available indicates that they have relatively small gut volumes compared with mammals. The high feeding rates and small gut volumes of birds typically result in short digesta retention times. Furthermore, intestinal mediated nutrient transport appears to be relatively low in small birds compared with mammals. But in some birds the absorptive capacity is vastly underestimated when based solely on measures of mediated uptake; direct measures of passive absorption show it to be much more important (1). Four avian species tested did not exhibit specific modulation of glucose transport (2). The observed low rates and lack of modulation of mediated transport pose challenging questions to the widely disseminated view that the transport capacity of apical sugar and amino acid transporters are matched to meet metabolic demands with some provision for a safety margin. The combination of short retention time, low intestinal surface area, and possibly low rates of nutrient absorption suggests that birds might operate close to the putative limit the digestive system sets on whole animal energy extraction. Indeed, when passerine birds eat fruit diets which are associated with short retention time, digestive efficiency is compromised (3). When challenged with changes in diet quality and quantity a general response of birds seems to be alteration in digesta flow and gut volume (2), which bring about nonspecific changes in overall nutrient absorption.

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4.3 COSTS AND BENEFITS OF ENDOTHERMY. Jared Diamond and Stephen Secor. Physiology Department, UCLA Medical School, Los Angeles, CA 90024.

Ambush-foraging snakes, such as pythons and rattlesnakes, are metabolically intermediate between endotherms and ectotherms. Such snakes consume huge meals (even exceeding the snake’s own mass) at long intervals (up to one year or more). When a fasted python swallows a rat, the snake’s metabolic rate rises by a factor of up to 40 and remains elevated for a week, until the rat is digested. This metabolic surge, costing about 30% of the rat’s energy content, represents the costs of digestion and of rebuilding metabolically active organs that are allowed to atrophy between meals. By incurring such start-up costs, snakes save the high maintenance costs of metabolically active organs during long fasts. Thus, ambush-foraging snakes are useful in identifying the costs and benefits associated with high metabolic rates.

4.4 ION HOMEOSTASIS AND ENDOThERMIC METABOLISM

P. L. Else, Biomedical Sci., Univ. Wollongong, 2500 Australia.

With the evolution of endothermy the cost of living went up. The same sized organism at the same body temperature cost 5 times more energy (1). Question is, what got expensive? One possible expense involves ionic homeostasis. Endothermic vertebrates have 2–5 times “leakier” cell membranes, and matched increased sodium pump metabolism compared to ectothermic vertebrates (2). Surprisingly, the numbers of sodium pumps appear not to have changed in the same tissues from ecto- to endotherms. Therefore the activity of each sodium pump is the molecular activity has increased several fold during the evolution of endothermy. At the same temperature (37°C) sodium pumps from endotherms can each turnover 2,800 ATP/min compared to 5,000 in endotherms in order to maintain ionic homeostasis. In support of this idea is the findings that sodium pumps taken from an ectotherm and placed in the membrane environment of an endotherm display higher molecular activities similar to those of endotherms. These results pose the idea that the evolution of endothermy involved changes in membrane composition that allowed membrane bound proteins to substantially increase their activities and supported the evolution of endothermy.

4.5 Mitochondrial function and endothermic metabolism

Martin D. Brand, Biochemistry Department, Cambridge University, U.K.

The bearded dragon, Amphibohorus vitticeps, is a lizard with the same body mass and preferred temperature as a rat, but (as is typical for ectotherms) with a five- to sevenfold lower standard metabolic rate than the endothermic mammal (1). The higher metabolic rate of the rat is due partly to an increase in the metabolically active internal organs like liver (1), but also to increased oxygen uptake by these organs: rat hepatocytes consume oxygen four times faster than lizard hepatocytes (2). Rat liver cells have about twice the content of mitochondria, and within the cells these mitochondria respire more of the rat’s energy content than the lizard’s liver mitochondria, and within the cells these mitochondria respire more of the rat’s energy content.

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Liposomes from mammalian liver mitochondria are more polyunsaturated and leakier to protons than those from reptiles Comp. Biochem. Physiol. B 108 (1994) 181-188
In order to understand the high metabolic rates of endotherms relative to ectotherms we have studied an agamid lizard, Pogona vitticeps and compared it to the laboratory rat. The large difference in energy turnover is partly due to the relative size of internal organs and partly due to cellular differences. Cells of endotherms have more mitochondrial membrane surface area and their mitochondrial membranes are leakier to protons. Liver cells are substantially leakier to Na⁺ and K⁺ with increased levels of sodium pump activity in the endotherm compared to the ectotherm. Phospholipids from rat tissues are significantly more polyunsaturated and less monounsaturated than equivalent lizard tissues. The proton conductance (both direct and facilitated) across liposomes made from lizard phospholipids is greater than across those made from rat mitochondrial phospholipids. This greater proton permeability is correlated with a more polyunsaturated bilayer. Its emerent differences. Further studies have suggested that this difference in the membrane fatty acid composition may be a fundamental difference between endotherms and ectotherms that can explain a number of other emergent differences.

**5.1 Calcium - regulator or regulated**

K. Simkiss, Univ. of Reading, United Kingdom

For most organisms the first sensation of life is a calcium wave that spreads around the newly fertilized egg and initiates development. This is calcium acting as a regulator. The last sensation of life is probably an uncontrolled calcium influx into the cell. This is the loss of calcium regulation. In between these two crucial events calcium acts both as a hormone, unique in that it is indestructible, and as a major "metabolite", unusual in that it must be carefully regulated as it is toxic to all cells.

In introducing this symposium the powerful tool of comparative physiology will be used to consider these processes. The talk emphasizes the conflicts between the regulation of mineral deposition and calcium fluxes at the cellular level and the regulatory and signalling functions of calcium at the cellular level.

**5.2 An Overview of Calcium Balance in Crustaceans.**

Michele G. Wheatley, Wright State University, Dayton OH 45435

This paper will discuss research since 1985 (1). Intermolt extracellular (EC) Ca is generally around 12 mM irrespective of habitat. In those crustaceans that exhibit negative Ca balance, EC Ca must originate from the exoskeleton/tissue. Branchial unidirectional influx is minimal but urinary reabsorption is significant in some freshwater (FW) species. EC Ca is tightly regulated in response to a range of environmental challenges but may drop when external levels drop below 25 mM. There is ongoing debate as to whether skeletal CaCO₃ is mobilized to compensate for severe systemic acidosis occasioned by exercise, hypercapnia, acid/aerial exposure etc. In terrestrial settings, where access to external HCO₃ is limited, EC Ca and HCO₃ often rise during acidosis but endoskeleton origin has not been definitively proven. Ca dynamics change greatly during the molting cycle. CaCO₃ is reabsorbed from the old cuticle which is shed; the new cuticle is mineralized partially with stored Ca but also with de novo sources. Postmolt calcification in aquatic species may involve passive mechanisms in 5W and active uptake processes that have been extensively reviewed in FW species. Postmolt unidirectional Ca uptake appears to be attributed to CA ATPase and/or a Ca/2H exchange, and is linked to HCO₃ and possibly Na⁺ uptake. Since the chemical reactions involved in mineralization are pH dependent, the process will only occur in a relatively alkaline microenvironment and will be impaired during external acidification. Terrestrial species (3) extensively recycle Ca by stirring it in moults and reingesting the shed remains; additional Ca is obtained from food and external water. While edysome is precipitated by the steroid hormone ecdysone, a direct link between ecdysone and Ca balance has not been established. (Supported by NSF 89-56412)

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Crustaceans form an unmineralized cuticle below their old one prior to each molt. A mechanism must exist to prevent mineralization of the pre-exuvial cuticle prior to the molt and then to rapidly calcify it after the molt. The pre-exuvial cuticle consists of the epi- and subcuticular layers and their structure has been described by numerous investigators. The exocuticle mineralizes immediately after the molt in a very predictable pattern. CaCO$_3$ first appears in hexagonal arrays perpendicular to the surface of the cuticle, and later forms distinct prisms in the exocuticle. Giraud-Guille et al. has suggested that these hexagonal arrays correspond to the lateral margins of the epithelial cells secreting the cuticle, and that the glycoproteins localized within interprismatic septa are a remnant of the cell coat. To characterize the glycoproteins associated with the areas of initial CaCO$_3$ deposition, Marlowe et al. used a battery of lectins to histochemically detect different sugar moieties within the cuticle. Marked differences were seen in the carbohydrate composition both spatially and temporally. Particularly, Concanavalin A and Jacalin became bound to the interprismatic septa immediately after ecdysis, roughly coinciding with the onset of mineralization. Similar temporal changes were observed in lectin binding to blots of EDTA-soluble glycoproteins extracted from pre- and postecdysial cuticle. Subsequent investigations have further resolved the period of glycoprotein transition to 1-3 hours postecdysis. These findings are consistent with the hypothesis that in situ modifications of carbohydrate moieties associated with cuticular glycoproteins can regulate mineralization.

5.5 TRANSERDERMAL CALCIUM TRANSPORT. P. Greenaway, R.M. Dillaman and R.D. Roer. UNSW, Sydney, 2052, Australia and UNC, Wilmington, NC, 28403.

During premoult large amounts of calcium salts are withdrawn from the old exoskeleton and transported across the epidermis to the haemolymph, for storage or excretion across the gills. After the molt, the new exoskeleton must be calcified and the water calcium salts are moved into the haemolymph from soft tissue stores and by uptake from the water across the gills and gut. From the haemolymph, they are transported across the epidermis and deposited in the new skeleton. The net fluxes involved at both moult stages may be very large. Transdermal calcium movement could follow paracellular or intracellular routes and physiological and ultrastructural evidence for these alternatives will be considered in premoult and postmoult animals for both gill and epidermal tissue. Intracellular transport routes require mechanisms for entry and exit of calcium from the cells plus a method of cellular transit which does not significantly elevate intracellular [Ca$^{2+}$]. The rapid reversal of direction of calcium fluxes at ecdysis requires equally rapid changes in the location orientation of these mechanisms.

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5.6 CELLULAR MECHANISMS OF CALCIUM TRANSPORT IN CRUSTACEANS. Dr. Gregory A. Ahearn (University of Hawaii, HI U.S.A.) and Dr. David W. Towle (Lake Forest College, IL U.S.A.)

This review will synthesize information presently available regarding Ca transport mechanisms in a variety of crustacean tissues including gill, hepatopancreas, antennal gland, and subcuticular epithelium particularly in relation to Ca mobilization and deposition associated with the molt cycle. Membrane level transport mechanisms to be addressed include: 1) Ca$^{2+}$-dependent ATPase, 2) Na$^+$/Ca$^{2+}$ antiport, and 3) Ca$^2+$/H$^+$ antiport. A low affinity Ca$^2+$ ATPase in homogenates of subcuticular epithelium demonstrates marked changes in activity associated with molting, but the precise role of this protein in trangsgenemic Ca$^{2+}$ movements in crustaceans is not clear. A Na$^+$/Ca$^{2+}$ antiporter has been demonstrated in nerve and muscle membranes of crustaceans and recent work has described the occurrence of this carrier in transporting epithelia of the antennal glands and hepatopancreas. Perhaps the most interesting of the likely Ca$^{2+}$ transport mechanisms is the Ca$^2+$/H$^+$ antiporter, an apparently alternative functioning of the electrogenic Na$^+$/H$^+$ exchanger previously described in three crustacean tissues (gill, antennal gland, and hepatopancreas). The Ca$^2+$/H$^+$ exchanger has been demonstrated in membrane vesicles from these tissues by two independent methods: 1) pH-sensitive Ca$^{2+}$ uptake and 2) Ca$^{2+}$ sensitive H$^+$ flux monitored with acridine orange.

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6.1 CHEMOSENSORY ROLE FOR Na+ CHANNELS IN AMPHIBIAN SKIN
S.D. Hillyard, Dept. Biology, Univ. of Nevada, Las Vegas, NV 89154

The primary route for water uptake by amphibians is via absorption across the skin. Many anuran species, especially those in the family bufonidae, have a region of pelvic skin that is specialized for water absorption. A variety of hormones including arginine vasotocin (AVT), and angiotensin II (AI1) have been shown to increase the rate of water uptake across the skin in vivo and in vitro. The increase in water permeability of the skin has been attributed to the insertion of water-conducting proteins into the apical membrane. Membrane capacitance measurements indicate that the apical membrane area of the pelvic but not the pectoral skin can be increased by treatment with AVT, however the increase is small and is not observed when an osmotic gradient exists across the skin, suggesting that the rates of vesicle insertion and retrieval may be similar. In order for physiological mechanisms to be utilized, toads must locate potential hydration surfaces and press their skin to them. This is accomplished by a behavior termed the water absorption response (WR). Toads give an increase in the expression of WR behavior. Thus, AI1 could serve to coordinate the physiological and behavioral mechanisms that optimize an animals' ability to rehydrate. The amphibian skin also transports Na+ and Cl- from dilute media to maintain the concentration of these ions in the body fluids as the animals absorb water across their skin. The rate of Na+ transport is limited by the number of active Na+ channels in the apical membrane and can be stimulated by AVT. We have recently found that amiloride-blockable Na+ channels serve a chemosensory function that allows toads to avoid WR behavior on surfaces made hypertonic with NaCl. Also, toads avoid hypertoneline KCl solutions by an amiloride-insensitive mechanism. Recordings from afferent neurons from ventral skin show a prolonged neural response when the skin is flushed with hypertonic solutions. Thus, ion channels in the epithelial cells of the skin appear to serve a sensory function in a manner similar to the taste buds of the lingual epithelium. (Supported by Nevada EPSCoR Proposal Development Grant)

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6.2 AMPHIBIAN AND REPTILIAN KIDNEYS: REGULATING THE FLOW.
Stanley D. Yokota. Department of Physiology, R.C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV 26506

The function of amphibian and reptilian kidneys has been considered to be controlled primarily through the extrinsic influence of arginine vasotocin (AVT), the congerual anti-diuretic hormone. AVT is released in response to hyperosmotic or hypovolemic stimuli and reduces urine output largely by decreasing the glomerular filtration rate (GFR). Mesotocin, a second neurohypophysial hormone, has been suggested to be a diuretic factor in amphibians. More recent evidence indicates that adrenergic effectors may also play important roles in the regulation of the kidneys, and that renal nerves are involved. In reptiles, roles for adrenergic and neural regulators have been implicated in whole animal and isolated kidney preparations. Norepinephrine elicits a glomerular diuresis at very low concentrations and antidiuretics at higher concentrations. Vasoactive intestinal peptide (VIP) causes a further diuresis in preconstricted isolated kidney preparations and is hypertensive in whole animals. In addition to these extrinsic influences, intrinsic mechanisms may also be important. The isolated perfused ophidian kidney is capable of substantial autoregulation of renal perfusion and GFR, and these autoregulatory responses are abolished in inhibitors of nitric oxide (NO) synthesis. NO appears to be an important modulator of peripheral vascular resistance as well as renal function, since in the whole animal, inhibitors of NO synthesis cause systemic hypertension. Supported by NSF BCR 9018611 and NIH 9318753.

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6.3 CELL AND MOLECULAR BIOLOGY OF WATER CHANNELS IN AMPHIBIAN BLADDEI3 AND MAMMALIAN KIDNEY.

Functional evidence has indicated that certain cell plasma membranes have high water permeability, including erythrocytes, kidney tubules and amphibian urinary bladder. Water channel proteins, which are members of the MIP protein family, have been cloned from mammalian and amphibian tissues. The first such water channel, CHIP28, is a 28 kDa integral membrane glycoprotein expressed in erythrocytes, kidney proximal tubule, and many fluid-secretory epithelia and endothelia. A tetrameric assembly of CHIP28 in the lipid bilayer (1) with 4 functional monomers has been established. While topology studies, hydrophy and amphipathic analyses of the tetrameric CHIP28 identified 4 to 8 membrane spanning domains. In addition, secondary structure analysis suggested mixed a-helix/β-sheet motifs (2). By homology cloning, several other proteins were obtained, including WCH 40, a water channel of kidney collecting duct. MIWC, a mucosal-insensitive water channel and GLIP, a stilbene-sensitive glycerol transporting protein. Localization studies showed different tissue distributions of these proteins in rat, apart from well defined regions in the kidney. The recent cloning of a CHlP28 like water channel from frog urinary bladder with ~80% identity to human and rat CHIP28 is indicative of functional similarity of water channels in mammals and amphibians (3). CHIP28 is believed to be a major constitutive water channel in kidney, suggesting the presence of yet another water channel, related to WCH 40, in amphibian bladder. The existence of water channel proteins in a variety of tissues and organs provides support for the notion that selective water transport occurs almost anywhere where required, data that could not be obtained by functional studies.

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7.1

PATTERNS OF GENE EXPRESSION DURING PHYSIOLOGICAL ADAPTATION. Hannah V. Cowley and J. N. Martin, Dept. of Comparative Biosciences, Univ. of Wisconsin, Madison and Deps. of Cell and Structural Biology, Univ. of Colorado School of Medicine, Denver.

Physiological adaptation on proximate or evolutionary time scales usually involves changes in protein expression and/or activity. Such changes can result from alterations at several points, from pre-transcriptional to post-translational steps. Examples from the comparative literature will be presented to generate discussion on the molecular basis for regulation of protein expression and the implications for biological adaptation. The ability of a protein to respond to rapid changes in the cellular or systemic environment may require the rapid response and reversal afforded by pre-transcriptional mechanisms. For example, during the dietary switch from ruminant to herbivorous modes in sheep, which involves dramatic changes in ruminal sugar concentrations, the abundance and activity of the Na+-glucose transporter is controlled primarily by pre-transcriptional or immediate post-transcriptional events. More stable, long-term changes in physiology may require less plastic mechanisms to increase protein activity, such as increased transcription of the corresponding mRNA. This appears to be the case for certain proteins that are upregulated just before and during hibernation by increasing their mRNA levels. Perhaps the ultimate level of physiological adaptation on a much longer time scale is the appearance of new genes during evolution that produce proteins that function more specifically and more efficiently for an organism's new adaptation. For example, the evolution of the lysozyme gene family involved gene duplications and divergences to new functions. The development of new genes, that can function at the low pH of gastric contents enabled forest-dwelling species to utilize the nutritional value of gut bacteria. The use of digestive lysozymes apparently has evolved independently in the ruminants, leaf-eating monkeys, and the hoatzin, a leaf-eating bird. Consideration of these three levels of regulation, i.e., pre-transcriptional events, changes in expression of existing genes, and development of new genes, may provide insight into the evolutionary (and physiological) costs and benefits of adaptation at different levels of molecular regulation.

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7.2

MODELING OF NEURAL CIRCUITS: WHAT HAVE WE LEARNED? Allen I. Selverston, Department of Biology, University of California, San Diego, CA 92093-0522.

The comparative approach to the study of neural circuits has successfully exploited the simpler nervous systems of invertebrates for over 25 years. Central pattern generators (CPGs) for rhythmic movements have been especially useful. Many CPG circuits have been described in detail and although they all use similar "building blocks", each CPG system has different synaptic arrangements. This is despite the fact that they all generate similar motor patterns. Recent studies indicate that invertebrate CPG neural circuits are actually in a dynamic state as a result of neuropeptide action, and neurons often switch from one circuit to another. The original goal of understanding how simple circuits work, and applying this knowledge to the formation of similar spatio-temporal patterns in the brain or spinal cord, has not yet been realized. Modeling is one way to help understand the input-output relationships of small CPG circuits, which are not intuitive, and how neuromodulators can alter their state. The basic question of whether or not our computational knowledge of small systems will be applicable to higher more complex nervous systems is still open.

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7.3

ACTUAL VERSUS IDEAL PERFORMANCE IN GAS EXCHANGE ORGANS. Frank L. Powell, Department of Medicine, University of California, San Diego, CA 92093-0322.

The diversity in structure of vertebrate respiratory organs results in a variety of different models of gas exchange, namely counter-current, cross-current and co-current (equivalent to ventilated pool or alveolar) models. The Goettingen school developed a theoretical framework to compare gas exchange efficiency in these models, and their actual performance in fish, birds, and mammals or reptiles, respectively (1). Predicted ideal efficiency of gas exchange, in terms of arterial Po2, is related in the following order: counter-current > cross-current> co-current. However, actual performance, in terms of arterial Po2, observed in nature, is remarkably similar. Theoretical and experimental studies show that this is not just because limitations, like heterogeneity and diffusion impairment, are greater in animals with more efficient models. Rather, the sensitivity of a model to heterogeneity and diffusion is proportional to its intrinsic efficiency (2,3).

This suggests the following general hypothesis: Intrinsically more efficient physiological systems, compared to less efficient models, operate farther from ideal performance levels in nature. This hypothesis can be tested by evaluating gas exchange conditions in different models, and considering other physiological systems (e.g. renal).

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CONFLICT AND COMPROMISE IN PHYSIOLOGICAL HOMEOSTASIS.

D.C. Jackson, Dept. of Physiological Sciences, Oregon State University, Corvallis, OR 97331.

The concept of the constancy of the internal milieu and the principle of homeostasis together constitute a fundamental paradigm of physiology. As formulated by Bernard, Cannon, and others (1), the emphasis has traditionally been on the maintenance of a stable extracellular state despite wide fluctuations in the external environment. A broader modern view, however, focuses on the intracellular compartment and, especially within a comparative physiological context, reveals homeostasis to be plastic and adaptive to stresses, both internal and external, to the organism, that often make regulation at a single fixed set of values either inappropriate, impractical, or impossible (2). The resting, awake, adult organism in an aqueous environment may no more be "normal" than the same organism asleep, hibernating, or in a cryptobiotic state as an encysted embryo. It is a central thesis of this presentation that these latter states can also be regarded as homeostatic and that organisms may regulate or maintain aspects of their internal and cellular environments quite differently depending on environmental, developmental, or temporal circumstances. In addition, it is proposed that an ordering of priorities exists for the organisms and that a similar ordering may also be revealed in the evolutionary emergence of regulated systems. Finally, it is proposed that at its most fundamental level, homeostasis requires the structural integrity of the cellular organelles and macromolecules and the functional integrity of isolating mechanisms that maintain special properties of the cellular fluid (3).

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EVENING PLENARY LECTURE

8.0 PROTEINS AND TEMPERATURE: LITTLE THINGS MEAN A LOT.

George N. Somero. Oregon State University, Corvallis, OR 97331.

The abilities of organisms adapted to widely different temperatures to sustain protein structure and function reflect the interplay of several "little things" of large importance. The net stabilization free energies of proteins are extremely low, of the order of only a few "weak" chemical bonds (Jaenicke, 1991). Thus, proteins are only marginally stable at physiological temperatures. Comparisons of homologous proteins from differently thermally-adapted organisms reveal that differences in average or maximal body temperature of only a few degrees can be sufficient to favor selection for modification of protein structure. Evolutionary fine-tuning of protein structure maintains the appropriate balance between stability and capacity for undergoing reversible changes in conformation during function. Only minimal changes in sequence are necessary to effect alterations in stability and kinetic properties (Jaenicke, 1991; Somero, 1995). Adaptive change commonly occurs outside of the active site regions. Additionally, the proper structure and function of proteins is influenced by the "micromolecules" of the cellular solution, notably, small organic solutes (osmolytes) and hydrogen ions (Somero and Yancey, 1995). Temperature-dependent pH regulation conserves key enzyme properties. Stabilizing osmolytes may enhance protein thermal stability; enzymes of certain hyperthermophilic archaebacteria are not inherently heat stable in vitro at 100°C, but are stabilized in situ by structure-stabilizing organic solutes. The combination of minor changes in protein sequence and adaptive changes in "micromolecules" of the cellular milieu allows proteins to function at temperatures ranging from the freezing point of seawater up to 110°C.

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MORNING PLENARY LECTURE

14.0 Evolution of Physiological Function: Insight on Endothermy in Fish

Barbara A. Block. Hopkins Marine Station, Stanford University

A new synergy is developing between modern phylogenetic analyses, physiology and biochemistry. The comparative method of studying animal physiology inherently provides a historical context for identifying the origin(s) and tracing the evolution of complex physiological traits. We have employed this approach to develop a better understanding of two distinct, but interrelated processes: the evolution of endothermy in fishes and the evolution of excitation-contraction coupling components in vertebrate muscle. Endothermy in fish is a complex trait with considerable interspecific and phenotypic variation. The existence of closely related ectothermic and endothermic species among scromboid fishes offers opportunities to identify the molecular and biochemical mechanisms underlying endothermy and also to assess the organellar and extracellular structure. Establishing an evolutionary framework for these analyses of scromboid fishes requires resolution of phylogenetic relationships among the species. We have done this by constructing molecular phylogenies for scromboid fishes and assessing their concordance with classically derived morphological phylogenies. Our current research exploits these independently derived phylogenies to examine the evolution of cranial endothermy in billfishes and the butterfly mackerel, and systemic endothermy in tuna. Among cranial endotherms we are examining the evolutionary transition of muscle from a contractile tissue to a thermogenic tissue. In tuna we are establishing the coupling between muscle of locomotion, aerobic capacity and endothermy. Results from studies of both groups are yielding insights into the association between ecological thermal niche and endothermic strategy.

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15.1 MORPHOLOGICAL DIVERSITY: IMPLICATIONS FOR RESPIRATORY CONTROL. W.K. Milsom, Department of Zoology, University of British Columbia, Vancouver, B.C., V61 2A9, Canada.

Attempts to analyze the evolution of central mechanisms involved in respiratory control are confounded by differences in the balance of the environmental, behavioural and morphological constraints placed upon the system in different species. Phylogenetically we see many trends. There is a switch from water to air as a respiratory medium, with some species utilizing both (bemyal breathers). There is a switch from gills to lungs for gas exchange with some species utilizing both, as well as the skin (1). There is a switch from \( \text{O}_2 \) to \( \text{CO}_2 \) as the major respiratory stimulus. There is a switch from a force pump driven by the buccal musculature to an aspiration pump driven by various muscles of the thorax and abdomen. There is an increase in metabolic rate and with this, a switch from intermittent to continuous ventilation and an increase in the partitioning of the lungs (2). The latter increases the resistance to lung inflation, increasing the cost of breathing and perhaps leading to the development of the diaphragm in mammals (3). Is the underlying control system in each vertebrate class different with similarities representing convergent evolution, or is the underlying control system for ventilation the same in all vertebrate classes with differences representing divergent evolution, dictated by these constraints? Newer data obtained from in vitro brainstem-spinal cord preparations show the strongest data to date that argue for the latter case.

15.2 A COMPARATIVE NEUROANATOMICAL STUDY OF RESPIRATORY CONTROL AND CARDIORESPIRATORY INTERACTIONS IN VERTEBRATES. E.W. Taylor, Univ. of Birmingham, United Kingdom.

The central nervous mechanisms controlling ventilation and cardiorespiratory interactions in vertebrates are relatively well described in fishes and mammals. Both groups are characterized by continuous rhythmic breathing; whereas the less well known air-breathing fishes, amphibians and reptiles often breathe discontinuously (Ballintijn, 1987). In fishes the respiratory muscles are all innervated by cranial nerves V to X plus the hypobranchial nerve, which incorporates anterior spinal nerves. Thus the respiratory motoneurons are located close to the respiratory rhythm generator in the brainstem, where they show a sequential topography which is reflected in their sequential firing during normal ventilation. The hypobranchial motoneurons are recruited during vigorous ventilation. This apparently primitive topography is retained in some air-breathing fishes and to some extent in amphibians, following development of the tetrapod lung and its associated structure, derived from the branchial skeleton.

Two populations of cardiac vagal motoneurons (CVM) have been identified neuroanatomically in the dorsal vagal nucleus (DVN) and in ventrolateral locations outside the DVN in the elasmobranch fishes. They have been designated separate roles in the reflex control of the heart and in centrally generated cardiorespiratory interactions (Taylor, 1992). Similarly two populations of CVM have been identified on functional grounds in mammals, although their central locations are as yet uncertain (Daly & Kirkman, 1989). There is evidence of progressive ventrolateral migration of vagal preganglionic neurons from the DVN during vertebrate ontogeny and phylogeny which may relate in part to control of cardiorespiratory interactions.

15.3 REGULATION OF SYNAPTIC STRENGTH WITHIN THE RESPIRATORY MOTOR NETWORK. Michael S. Dekin, Dept. Medicine, R.W. Johnson Medical School, UMDNJ, New Brunswick, NJ 08803-0019

Neurochemical modulation of ion channel activity underlies plasticity in neuronal circuits such as that controlling rhythmic breathing movements in vertebrates. Modulation of postsynaptic properties can alter the spatiotemporal pattern of neuronal firing activity while presynaptic modulation is an important mechanism for adjusting the strength of synaptic connections between neurons within a circuit. In invertebrates, neurochemical modulation of ion channel activity has been shown to underlie both motor learning and circuit reconfiguration. Learning leads to the strengthening (or weakening) of the motor output (ref #1) while reconfiguration allows the circuit to display new patterns of activity or even participate in other behaviors (ref #2). Recent in vitro studies have demonstrated that many of the ion channels responsible for circuit plasticity in invertebrates are also found in neurons controlling rhythmic breathing movements in vertebrates. One example is an \( \alpha \)-like \( \kappa \) channel similar to that responsible for neurohormone release in the marine mollusc, \textit{Aplysia} (ref #3). In vertebrate respiratory neurons, this channel is activated by \( \gamma \)-aminobutyric acid acting at its \( \beta \) receptor. The activity of this channel is also modulated by thyrotropin-releasing hormone via a protein kinase A dependent phosphorylation pathway. The role of this channel in regulating the strength of the synapse between premotor respiratory neurons will be discussed. (Supported by NIH Grants HL40369 and HL02314 and a UMDNJ Foundation Grant).

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15.4

**FUNDAMENTALS OF CENTRAL RESPIRATORY RHYTHM AND PATTERN FORMATION.**

Jack L. Feldman, Department of Physiological Science, UCLA

The brain is vigilant in control of breathing, responsible for the regulation of blood oxygen and carbon dioxide adaptable over an order of magnitude range in metabolic demand, wide ranges of posture, body movements, emotions, compromises in muscle or cardiovascular function, from birth till death without lapses beyond a few minutes. It must make efficient use of the respiratory musculature, for the metabolic cost of inactivity, integrated over time, is considerable. Moreover, serious respirovascular muscle failure must be avoided to prevent insufficiency, especially during and following extreme exertion or with disease. Our current understanding of how the brain controls breathing is fragmentary. In the past decade, a solid foundation has been established that may serve as the basis for resolution. I will discuss several hypotheses:

- The PreBötzinger Complex, in the rostral ventrolateral medulla, is the brainstem locus for rhythm generation.
- Bursting pacemaker neurones are the kernel for generation of respiratory rhythm.
- Respiratory rhythm is generated by a hybrid pacemaker network.
- Cholinergic is the primary fast neurotransmitter in this network.
- Many neurotransmitters modulate respiratory pattern by both pre- and postsynaptic actions.
- Several key neurotransmitters affect respiratory pattern by modulating the conductance of various potassium channels.

15.5

**CHEMORECEPTION AND RHYTHM GENERATION IN LOWER VERTEBRATES.**

Remmers, J.E., Kawasaki, H., Kimura, N., Kogo, N., Perry, S.F. Dept. of Medical Physiology and Medicine, University of Calgary, Calgary, Alberta, CANADA T2N 4N1

Amphibians provide an opportunity to explore primitive neural mechanisms responsible for respiratory chemosensitivity and rhythmogenesis. To this end, we have developed and validated a fictitious breathing in vitro preparation of the brainstem of larval (R. catesbiana) and adult (R. catesbiani and R. pipiens) frogs. These preparations exhibit rhythmic, alternating, coordinated motor outputs from cranial nerve (CN) roots innervating gill (CN VII and VIII), oesophageal (CN V, VII, IX, SN II) and laryngeal (CN X) muscles. This bursting activity was linked to buccal and pulmonary ventilation of the intact animal via partially reduced, intermediate preparations which showed activities in nerves to identified respiratory muscles resembling those of the completely isolated preparation. In adults and tadpoles, a rhythm generator, located bilaterally across cranial nerves V and IX responded to changes in superfused pH. Fictive lung ventilation in the adult was arrested by the non-NMDA and GABA_B blockers, CNQX bicuculline, and by opioid agonists. Rhythmic bursting persisted after glycergic blockage by strychnine, but reciprocity was eliminated and the burst shape changed from augmenting to decrementing. In the tadpole brainstem, bicuculline increased respiratory frequency and amplitude. The results reveal the operation of a central respiratory chemoreceptor and rhythm generator dependent on non-NMDA neurotransmission. GABA_B neurotransmission is essential in the adult but not in the larva. (Supported by MRC grant #MA9719)

15.6

**Brain and Breathing: Snail sets the pace.**

Naweed I. Syed, Department of Anatomy and Physiology, Respiratory Research Group, Faculty of Medicine, The University of Calgary, 2220 Hospital Drive NW, Alberta, Canada, T2N 4N1

The lack of fundamental respiratory knowledge regarding neural control of vertebrate breathing owes its existence to the complexity of the behavioral repertoire and the intricate nature of respiratory neural networks in the snail. Our strategy for studying respiratory rhythmogenesis is to develop an invertebrate model system which exhibits relatively simple and the underlying respiratory network is identifiable and accessible for neurophysiological analysis. In our quest, we use the freshwater snail, *Lymnaea stagnalis*, to explore fundamental mechanisms underlying respiratory control. Lymnaea is a bimodal breather, i.e., it uses either cutaneous gas exchange with water, or lung gas exchange with the gaseous atmosphere. This fresh water snail employs aspirational lung breathing which is hypoxia-driven. We have described respiratory control. Lymnaea exchange with water, or lung gas exchange with the gaseous atmosphere. This fresh water snail employs aspirational lung breathing which is hypoxia-driven. We have described respiratory control. Lymnaea exchange with water, or lung gas exchange with the gaseous atmosphere. This fresh water snail employs aspirational lung breathing which is hypoxia-driven. We have described respiratory control. Lymnaea exchange with water, or lung gas exchange with the gaseous atmosphere.

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This paper establishes the existence of a central respiratory chemoreceptor in the frog sensitive to changes in pH and P_CO2.

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Neuron 8, 1992, 767-774
16.1

STRUCTURE AND REGULATION OF THE A ADRENERGIC-ACTIVATED Na+H+ ANTIPORTER OF TROUT RED CELLS.

R. MOTAILS, R. BORGREE, H. GUIZOUAUX and A. R. GARCIA-ROMEOU

Laboratoire Jean Moats, Département de Biologie Cellulaire et Moléculaire du C.R.S.A., BP 68, 06250 Villefranche-sur-Mer, France

The Na+/H+ exchanger found in the membrane of a nucleated erythrocyte (trout red cell) is an interesting isomer of the human antipporter NHE1: 1) it does not reside in the intercellular pHi; 2) it is activated by adrenergic agonists whereas the other isomers are either insensitive or activated by CAMP; 3) its activation is rapidly followed by its desensitization; 4) its activity is conserved by molecular oxygen, a property related to its physiological function. This system, called BNHE, exhibits a high degree of homology with the NHE1 antipporter transmembrane domain while the cytoplasmic domain is more divergent. It is responsible to restore the functional feature of the trout red cell antipporter (activation by CAMP and PKC activators) when expressed in antipporter-deficient hamster fibroblasts. An examination of the sequence of the cytoplasmic regulatory domain of BNHE reveals two very close consenm sites for PEFK-A which are not present in the human NHE1. A got insight into the role of these PEFK-A sites and other sites of the cytoplasmic domain, a set of punctual mutations and deletion mutations has been generated. These effects form expressed in antipporter-deficient fibroblasts reveal that 1) activation by cAMP mimics the activity of the PKA sites; 2) a deletion of the cytoplasmic domain which contains PEFK-A consensus sites abolishes the CAMP activation but does not impair the kinase-C mediated activation; 3) a chimeric NHE1 transmembrane domain/BNHE cytoplasmic domain is fully activated by CAMP. These results emphasize the notion that the cytoplasmic domain of the antipporter, although not essential for basal activity, is crucial to mediate the various hormonal response. Moreover, evidence the different signaling pathways do not necessarily converge on "integral" kinases such as MAP kinase as previously suggested for NHE1 activation. The kinetic results obtained in presence of phosphatase inhibitors lead us to propose a model for activation and desensitization of BNHE. It seems likely that regulation of the antipporter involves a recycling mechanism.

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   Regulation of the Na+/H+ exchange activity by recruitment of new Na+/H+ antipporters. Effect of caycalin A, a phosphatase inhibitor.
   An. J. Physiol. (cell physiol) in press.

16.2

K FLUX PATHWAYS IN TROUT RED CELLS: REGULATION BY OXYGENATION, CELL VOLUME AND PROTEIN PHOSPHORYLATION.

A. R. CoeRNS and Y. Weaver

Department of Environmental and Evolutionary Biology, University of Oxford, OX1 3PT, U.K.

Trout red cells possess two powerful K flux pathways, the KCI cotransporter and a Cl-independent K pathway. Activation of the first by oxygenation or adrenergic stimulation and the latter by hypotonic swelling leads to net KCI efflux and cell shrinkage, respectively (1,2). The protein phosphatase inhibitors, carycalin A and okadaic acid, inhibit the oxygenation-activated cotransporter but not the hypotonically-activated KCl-co-transporter (3). This clamped activity was volume-dependent indicating that the KCl cotransporter is activated by a complex cascade regulates K flux pathways (3). We suggest that staurosporine acts on a separate kinase which controls the activity of the carycalin A-sensitive phosphatase. This indicates that there is a certain permeability to monovalent cations. The Na+K+ exchanger found in the membrane of a nucleated erythrocyte (trout red cell) is an interesting isomer of the human antipporter NHE1: 1) it does not reside in the intercellular pH; 2) it is activated by adrenergic agonists whereas the other isomers are either insensitive or activated by CAMP; 3) its activation is rapidly followed by its desensitization; 4) its activity is conserved by molecular oxygen, a property related to its physiological function. This system, called BNHE, exhibits a high degree of homology with the NHE1 antipporter transmembrane domain while the cytoplasmic domain is more divergent. It is responsible to restore the functional feature of the trout red cell antipporter (activation by CAMP and PKC activators) when expressed in antipporter-deficient hamster fibroblasts. An examination of the sequence of the cytoplasmic regulatory domain of BNHE reveals two very close consenm sites for PEFK-A which are not present in the human NHE1. A got insight into the role of these PEFK-A sites and other sites of the cytoplasmic domain, a set of punctual mutations and deletion mutations has been generated. These effects form expressed in antipporter-deficient fibroblasts reveal that 1) activation by cAMP mimics the activity of the PKA sites; 2) a deletion of the cytoplasmic domain which contains PEFK-A consensus sites abolishes the CAMP activation but does not impair the kinase-C mediated activation; 3) a chimeric NHE1 transmembrane domain/BNHE cytoplasmic domain is fully activated by CAMP. These results emphasize the notion that the cytoplasmic domain of the antipporter, although not essential for basal activity, is crucial to mediate the various hormonal response. Moreover, evidence the different signaling pathways do not necessarily converge on "integral" kinases such as MAP kinase as previously suggested for NHE1 activation. The kinetic results obtained in presence of phosphatase inhibitors lead us to propose a model for activation and desensitization of BNHE. It seems likely that regulation of the antipporter involves a recycling mechanism (5).

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   An. J. Physiol. (cell physiol) in press.

16.4

Volume-Sensitive Organic Osmolyte Transport Through a Cl-Channel.

J. Clive Ellory, Jo-Ann Lancaster and Uni Katz

Dept. of Physiology, University of Oxford, OX1 3PT, U.K.

Efflux of organic solutes makes a major contribution to cell volume regulation in response to hypotonic shock. Using flounder and Xenopus erythrocytes and trout hematocytes we have shown that not only the paradigm organic osmolyte taurine, but glucose, uridine and inositol, but not sucrose, lysine or glutamine are effective permeants in this pathway.

There is also significant transport of choline, and it is likely that, as for CI-channels there is a certain permeability to monovalent cations. Transport is inhibited by large anionic molecules. In animal tissues, CI-Channels blockers (NPPB), KCI co-transport inhibitors (DIOA) and Band 3 inhibitors (influrame, DIDS). Enhanced efflux only occurs when volume changes exceed 25%, i.e. there is a distinct threshold of activation. Raising [Cl] does not promote this pathway and it is likely that AA metabolites/leucotrienes are the signalling pathway involved.

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16.5

ADAPTIVE RESPONSES OF RED CELLS TO HYPOXIA AND HYPERCAPNIA.

Mikko Nikinmaa, Department of Zoology, FIN-00014 University of Helsinki, Finland

This review focuses on the responses of fish erythrocytes to hypoxia/hypercapnia, because especially the freshwater environment is characterized by large fluctuations in oxygen and carbon dioxide tensions. The red cell responses facilitate oxygen loading to gills by producing a leftward shift of the oxygen equilibrium curve. This is achieved via a reduction of cellular ATP concentration, an increase in intracellular pH or a dilution of hemoglobin within the cell. In teleost fish the first two mechanisms predominate, whereas in lampreys, the hemoglobin of which are insensitive to organic phosphates, the latter two mechanisms are important. In hypoxy-exposed teleost fish the erythrocyte pH is rapidly increased by adrenergic activation of the sodium/proton exchange. At the onset of hypoxy catecholamines are liberated into the blood stream, and the number of functional β-adrenergic receptors increases. Binding of catecholamines to the receptor increases cellular cAMP levels and activates the sodium/proton exchanger which has a higher turnover rate in deoxygenated than in oxygenated erythrocytes. The activation of sodium/proton exchange also increases the cell volume. The cell swelling depends on the relative rate of net sodium influx and potassium, chloride (and uric acid) efflux. The potassium efflux pathways, probably involved in cell swelling, are also communicated by the other related transmembrane pathways. Inhibiting the sodium/proton exchange by reduction in cellular cAMP concentration, they do not cause the hypoxygen-induced reduction of ATP levels. As yet, the pathways regulating cellular ATP concentrations during hypoxia acclimation are not known.

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16.6

THE INTERACTIVE EFFECTS OF STRESS, ADRENERGIC RECEPTORS AND RED CELL ADRENERGIC RESPONSES. Steve F. Perry & Scott D. Reid. Dept. of Biology, University of Ottawa, Ontario, Canada, K1N 6N5

The teleost red blood cell contains at least three populations of β-adrenergic receptors distributed within the cytosol and on the cell surface. The high-affinity surface receptors are linked to cAMP formation and the resultant cellular adrenergic responses. The numbers of these receptors can be increased rapidly by recruitment of the cytosolic receptor pool thereby enhancing the adrenergic responsiveness of the red cell. Differing numbers of cell surface receptors may also partially explain the marked inter-specific variability in the responsiveness of teleost red cells to catecholamines.

Repeated stress can significantly alter the red cell β1 receptor populations owing to the effects of the glucocorticoid and catecholamine stress hormones. Elevated plasma levels of cortisol causes a pronounced increase in the size of the cytosolic receptor pool leading to enhanced adrenergic responsiveness during acute stress as these additional receptors are mobilized to the cell surface. Conversely, chronic elevation of the catecholamines, adrenaline and noradrenaline, decreases the number of cell surface β1 receptors (down-regulation). During actual repeated stress (e.g. daily handling), cortisol and catecholamine levels are both elevated and presumably influence the red cell receptors in opposing ways. The net effect, however, is a significant reduction in cell surface receptor numbers indicating that the catecholamine elevation exerts the predominant effect.

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Perry, S.F., Reid, S.D. β-adrenergic signal transduction in fish: Interactive effects of cortisol and catecholamines. Fish Physiology & Biochemistry 11, 1993, pp. 195-203

A recent review summarizing the interactive influences of acute and chronic stresses on red cell adrenergic responses


This review chapter focuses on the control and consequences of catecholamine release in fishes


A thorough review on the unique mechanisms employed by nucleated red cells to regulate hemoglobin-oxygen binding

17.1

Stabilization of proteins during freezing and drying. Thomas J. Anchordoguy and John F. Carpenter. School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80220

A wide variety of compounds will protect labile proteins during freezing. These include sugars, amino acids, polyols, methylamines, synthetic polymers, other proteins and even certain inorganic salts. Protein cryopreservation can be explained by the same universal mechanism that Timasheff and Arakawa have defined for solute-induced protein stabilization in nonfrozen, aqueous solution. The solutes are preferentially excluded from the protein, increase the protein's chemical potential and make it more thermodynamically unfavorable for the protein to denature. In contrast, disaccharides are most effective at protecting labile enzymes during freeze-drying or air-drying. Thus, protection of proteins against dehydration stress appears to be fundamentally different from cryopreservation. We have found, using solid-state Fourier transform infrared spectroscopy, that the hydrogen bonding of the sugar to the dried protein is necessary for protein preservation. Also, we have found that labile proteins, dried in the presence of sucrose, retain their native secondary structure in the dried solid. Thus, the mechanism by which sugars preserve enzyme activity during freeze-drying and rehydration is by preventing unfolding during the freezing and drying stage. Finally, to investigate the role of quaternary structural alterations in protein damage during freezing and drying, we have used formation of hybrids from lactate dehydrogenase isozymes as an indicator of reversible dissociation. We have found that stabilizers must inhibit freezing-induced dissociation to preserve the protein during freeze-thawing and freeze-drying.

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16.6

ANHYDROBIOLOGY

Perry, S.F., Reid, S.D. β-adrenergic signal transduction in fish: Interactive effects of cortisol and catecholamines. Fish Physiology & Biochemistry 11, 1993, pp. 195-203

A recent review summarizing the interactive influences of acute and chronic stresses on red cell adrenergic responses


This review chapter focuses on the control and consequences of catecholamine release in fishes


A thorough review on the unique mechanisms employed by nucleated red cells to regulate hemoglobin-oxygen binding

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MODELS FOR ANHYDROBIOSES: STUDIES ON CONFORMATIONAL STATES OF DRY PROTEINS. Steven Prestretski, Alza Corp., Palo Alto, CA 94304.

The conformation of a protein is essential to its biological activity. Thus, maintenance of the proteins conformation during dehydration would seem essential to stability of dried proteins. To explore the relation between protein structure and storage stability in the dried state, we have examined the conformation of proteins in the aqueous and dried state using Fourier-transform infrared spectroscopy. Various compositions were tested for their capacity to preserve the native conformation of proteins upon lyophilization. In our model, results demonstrate a direct correlation between preservation of the native (aqueous) structure during dehydration and long-term stability in accelerated stability studies. Retention of the native structure resulting in enhanced stability to physical degradation (i.e., aggregation) and chemical degradation (i.e., covalent cross-linking). Formulations which led to unfolding during dehydration also indicated decreased stability and the loss of stability appears strongly related to the degree of unfolding. Additional studies have demonstrated that the composition of the rehydration medium can also have significant impact on the recovery of native, active protein. Potential mechanisms for degradation will be discussed.

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MODELS FOR ANHYDROBIOSES: PRESERVATION OF LIPID BILAYERS DURING DRYING. Lois M. Cryer and John H. Cryer, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616.

Based on the accumulation of evidence which showed that anhydrobiotic organisms contained significant amounts of sugars (especially sucrose and trehalose) when dry, we developed a simple model system to investigate more directly the interactions of sugars with phospholipid bilayers. Using Fourier Transform Infrared Spectroscopy (FTIR), differential scanning calorimetry (DSC), resonance energy transfer, and leakage of trapped solutes, we studied the effect of sugars on bilayer fusion, phase transitions, and leakage from unilamellar and multilamellar liposomes. Prevention of fusion could be obtained by as little as 0.2-0.4 g trehalose/g lipid, but prevention of leakage from fluid liposomes (POPC or egg PC) required about 1 g trehalose/g lipid. DSC and FTIR showed that trehalose at about 1 g/g lipid lowered the phase transition of dry phospholipids to below the hydrated transition temperature. For fluid lipids, such as those found in biological membranes, this lowering of $T_m$ means that the membrane does not undergo a leakage-inducing transition during the lytophytic phase change of rehydration at room temperature. More rigid lipids, with a hydrated phase transition temperature above room temperature require only enough sugar present to prevent fusion in order to maximize solute retention. FTIR also produced evidence for a direct interaction between the phosphate of the bilayer and hydroxyl groups of the sugar. This interaction, which spreads the headgroups and maintains the lipid in a liquid crystalline state while dry, is necessary for bilayer preservation. More recent experiments have demonstrated that it is necessary that the phospholipid:sugar preparation remain in a vitrified state during all stages of drying in order to preserve their integrity, but that vitrification alone is not sufficient for preservation.

REFERENCES:

MODELS FOR ANHYDROBIOSES: STABILIZATION OF BIOLOGICAL MEMBRANES DURING DRYING. John H. Cryer, Samuel D. Leslie, and Lois M. Cryer, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616.

Previous presentations in this symposium established that disaccharides are particularly effective at stabilizing proteins and phospholipid bilayers during drying. In this paper we will show that the same sugars stabilize intact biological membranes during drying as well. When vesicles of sarcoplasmic reticulum were dried without sugar they fused to form larger vesicles, underwent lateral phase separation of protein and lipid components, and lost all biological activity. When the same membranes were dried with trehalose, morphological evidence for damage was lacking, and upon rehydration these membranes showed normal biological activity. The mechanism of preservation is similar to that seen in liposomes; the sugars depress lipid phase transitions in the dry membranes, maintaining them in a fluid phase even when they are dry. Studies with infrared spectroscopy showed that membrane proteins are maintained in their native conformation when the membranes are dried with the sugars, but are denatured to random coil if the membranes are dried without the sugars. Comparable results have been obtained with intact cells. Lipid phase transitions in yeast cells and bacteria dried without trehalose are elevated, but are depressed to near those of hydrated cells if trehalose is present. Proteins in bacteria dried without trehalose were irreversibly converted to random coil, but with increasing amounts of trehalose present conformational state was maintained near that of the hydrated cells. Supported by grants NIH-9318851 from the National Science Foundation and NAVO 179 from the Office of Naval Research.

Seed, embryos and pollen tend to accumulate sucrose and oligosaccharides upon maturation drying. Generally they are tolerant to desiccation. Cultured somatic embryos can be evoked to become desiccation tolerant, but below a total saccharide content of 10% of the dry weight, there are no survivors of dehydration. Such desiccation sensitive individuals leak cytosolic solutes during discontinuous imbibition. Employing FTIR, shifts of the symmetric CH vibration band were noticed in intact pollen in relation to its moisture content. On account of such behavior we assigned the CH absorption band around 2850 cm^-1 to phospholipids in the membranes (1). The calculated Tc ranged from -6°C in hydrated pollen to 32°C in very dry pollen. Dried isolated membranes had a Tc of 60°C, which was reduced to 20°C in the presence of sucrose, the major label in the carbohydrate in the pollen (2). We conclude that sucrose effectively reduces the rise of Tc of membrane phospholipids. In situ dehydration above this level still causes leakage. Thus, it may provide desiccation tolerance. Certain seeds tested also showed reduced rise of Tc with drying. Aging leads to imbibitional leakage of solutes. This may be discussed by the observed gradual rise of Tc with time, which reaches room temperature for hydrated membranes (3). The conformational status of proteins was not affected during dry aging. The rate of dry aging of various desiccation tolerant plant organs in relation to the protecting carbohydrates species involved will be discussed.

17.6

Studies on intact anhydrobiotes: a role for sugar transport in stabilization of membranes and proteins

Pedro Soares de Araujo1 and Anita D. Panek2

1Departamento de Bioquímica, Instituto de Química, UFRJ, Rio de Janeiro, Brazil; 2Departamento de Química, Instituto CT, R. A, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941, Brazil.

In the yeast Saccharomyces cerevisiae, a model for eukaryotic cells, trehalose plays an important protective role for its resistance to freezing, heat shock and desiccation (1). This protection depends on the presence of the sugar on both sides of the yeast plasma membrane (2) and since there is no free trehalose in nature, trehalose transport is of utmost importance to anhydrobiotes. These results are in perfect agreement with those obtained for model systems, like liposomes, or soluble enzymes during dehydration (3). In yeast cells this permease is a specific protein entity albeit as other disaccharide transporters it shows a proton symport mechanism. Its synthesis depends on the growth phase of the cell and is induced by growth on maltose or trehalose as sole carbon source. h isolated plasma membrane vesicles trehalose transport is vectorial with the pH gradient thus providing a means for the maintenance of pH homeostasis and maintaining protein stability (4). Genetic evidence indicates that the expression of the transporter is regulated by the same gene that regulates maltose transport in stabilization of membranes and proteins. The calculated Tc ranged from -6°C in hydrated pollen to 32°C in very dry pollen. Dried isolated membranes had a Tc of 60°C, which was reduced to 20°C in the presence of sucrose, the major label in the carbohydrate in the pollen (2). We conclude that sucrose effectively reduces the rise of Tc of membrane phospholipids. In situ dehydration above this level still causes leakage. Thus, it may provide desiccation tolerance. Certain seeds tested also showed reduced rise of Tc with drying. Aging leads to imbibitional leakage of solutes. This may be discussed by the observed gradual rise of Tc with time, which reaches room temperature for hydrated membranes (3). The conformational status of proteins was not affected during dry aging. The rate of dry aging of various desiccation tolerant plant organs in relation to the protecting carbohydrates species involved will be discussed.

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FROM MYXINE TO MAN: THE PHYSIOLOGY OF BLOOD VOLUME REGULATION

10.1

PHYSICAL FACTORS AND RENAL EXCRETION–ROLE IN BLOOD VOLUME REGULATION. Allen W. Cowley. Medical College of Wisconsin, Milwaukee, WI, 53226

Blood volume (BV) in mammals is controlled by the complex interaction of physical factors and reflex-hormonal systems which determine the rate of Na+ and H2O excretion. This presentation introduces studies carried out to determine the role of physical factors (renal arterial hydrostatic pressure (RAP) and plasma colloidal osmotic pressure (COP)) in the short and long-term control of BV. COP: Expansion of the extracellular space with an increase in blood volume (BV) is associated with the CVP and COP in the redistribution of body fluids. A rapid RAP reduction of 20% decreases the BV. This is accompanied by renal responses dependent on the diuresis mechanism. Final steady-state fluid balance appears to be achieved by the pressure-diuresis mechanism.

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**18.2**

**EXTRACELLULAR FLUID AND BLOOD VOLUME HOMEOSTASIS IN SALWATER HAGFISH AND ELASMOBRANCHS.** Sambhala Banerji and Stanley D. Yokota. Dep't of Physiology, Univ. Oklahoma HS, Oklahoma City, OK, 73109 and West Virginia Univ., Morgantown, WV 26506.

Both hagfish and elasmobranchs maintain their body fluid osmolality slightly hypoosmotic to the marine environment, therefore they both are subjected to饮水 to compensate for hypovolemic stress appears to be lymph mobilized via lymphatic systems.

1. It is not clear how much of the albumin BV is due to a highly protein-permeable
2. vertebrates, while labeled albumin produces considerably higher values (83 mlakg-
3. 4. Distribution (30-45 mlakg-1) are consistently lower than those reported for other
4. Systems. While the ability of fish to regulate plasma and tissue osmolarity in
5. 2. Fish and not transcapillary oncotic uptake. Upon depletion of lymphatic reserves,

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role of the renin-angiotensin system, kallikrein-kinin system, and atrial natriuretic peptides.

**18.3**


Ryn fish thrive in both hydrating, salt repleting (freshwater) and
dehydration, salt-loaded (seawater) environments. Euryhaline species can be
adapted to either environment and are potentially valuable models with which to
examine processes involved in regulation of intravascular and interstitial fluid
compartments. While the ability of fish to regulate plasma and tissue osmolarity in
hyper- or hypotonic environments has been extensively characterized, the
size, much less control, of fluid volume in either environment, is not understood.

Indicator dilution estimates of blood volume (BV) based on labeled red
cell distribution (30-45 mlakg-1) are considerably lower than those reported for other
vertebrates, while labeled albumin produces considerably higher values (83 mlakg-
1).

It is not clear how much of the albumin BV is due to a highly protein-permeable
vascular endothelium or to the presence of a second, red cell inosvascous, vascular
compartment. This secondary system, apparently unique among vertebrates,
arises from primary systemic arteries in the form of a myriad of small arterioles that
conducts from primary systemic arteries to the circulatory tree (89 mlkg-1).

It is not clear how much of the albumin BV is due to a highly protein-permeable
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compartment. This secondary system, apparently unique among vertebrates,
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conducts from primary systemic arteries to the circulatory tree (89 mlkg-1).

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18.5 BLOOD VOLUME REGULATION IN REPTILES. H.B. Lillywhite.
University of Florida, Gainesville, FL 32611.

Reptilian blood volumes range between 4-13% of body mass, with extremes represented by marine snakes. Studies of snakes and turtles have demonstrated substantial shifts of fluid volume between vascular and interstitial compartments, suggesting that resistance to transcapillary fluid movement is low. As a consequence, snakes are able to maintain arterial pressures during graded hemorrhage of 63-120% of the initial blood volume, during which 20-71% of the hemorrhaged volume is replaced by transcapillary shifts of extravascular fluids (1). Such volume compensation appears to be attributable to strong reflex vasocostriction in porphyrin tissues, which results in increased pre-to postcapillary resistance ratio and attendant fall in capillary pressure. The source of extravascular fluid entering the vascular space is entirely extracellular during acute volume shifts, but intracellular fluid may enter the blood within 2 h following moderate levels of hemorrhage (2). Translocation of plasma from blood to interstitial occurs in response to exercise, elevated blood pressure, and gravitational pooling of blood during upright posture. In spite of the liability of blood volume, reptiles are able to regulate hemodynamic and respiratory functions effectively during hypovolemic challenges (3). Long-term regulation of blood volume is probably similar to that in mammals, but remains to be investigated.

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18.6 HORMONAL REGULATION OF BLOOD VOLUME IN BIRDS
Yoshiro Takei, Ocean Res. Inst., Univ. Tokyo, Tokyo 164, Japan

Terrestrial homeothermal species are constantly faced with a need to conserve water to maintain blood volume. Conditions are severe in birds which have higher body temperature and higher degree of activity than mammals. In discussing the regulation of blood volume, both water and sodium have to be taken into account, because if water alone is given to dehydrated birds it is soon excreted, but if isotonic saline is given blood volume is maintained. There is evidence to suggest that uroptotic receptors to monitor extracellular fluid volume are located in the extracellular, interstitial compartment in birds.

Thirst is induced principally by angiotensin II (ANGII), whereas ANGII and aldosterone (ALD) act synergically to induce sodium appetite in birds. The cloaca serves as an important osmoregulatory organ in addition to intestine in birds, and ALD stimulates cloacal absorption of sodium. Atrial natriuretic peptide (ANP) is known to antagonize every aspect of ANGII effect in mammals, but intracranial mammalian ANP is dipogenic in the quail. ANP has not been identified in birds, although B-type (BNP) and C-type natriuretic peptides were sequenced in the chicken.

Arginine vasotocin (AVT) and ALD are water- and sodium-retaining hormones in birds as in mammals. Subpressor doses of ANGII are antidiuretic and antinatriuretic in birds, but most of its effects seem to be mediated by its action on AVT and AVT release. Chicken BNP is diuretic and natriuretic in birds, and it inhibits ALD release but not AVT release. The nasal salt gland serves as another osmoregulatory organ in some birds, whose secretion is most potently inhibited by ANGII and stimulated by chicken BNP.

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19.1 CALCIUM REGULATION IN AQUATIC VERSUS TERRESTRIAL POIKILOTHERMIC VERTEBRATES. James C. Fenwick. Dept. Biology, Univ. Ottawa, Ottawa, Ontario, Canada, KIN 6N5

Because of the myriad of biochemical and physiological effects of calcium in all vertebrates must regulate the concentration of calcium in their extracellular fluids. But the control is, in general, realised in two intrinsically contrasting ways. Both fish and primarily aquatic amphibians, possess competent systems for accessing the effectively limitless supply of calcium dissolved in their ambient medium. Indeed, these systems are so effective that the primary physiological controls are directed towards the prevention of hypercalcemia. Conversely, terrestrial vertebrates acquire calcium only through their diet and thus face alternating periods of high calcium intake and no calcium intake. Consequently, they must have endocrine controls which can prevent post-prandial hypercalcemia while simultaneously ensuring calcium storage when calcium is available and other hormonal controls which can mobilise previously stored calcium when it is at a premium. In short, calcium homeostasis in bofy bird is dominated by the antihypercalcemic hormone, calcitonin, which operates primarily by reducing calcium uptake through the gills. Wholly aquatic amphibians appear to be calcitonin, another hypercalcemic hormone. Conversely, calcium regulation in terrestrial amphibians, and the reptiles, follows the avian and mammalian model and is under the primary control of the hypercalcemic hormone, parathormone. This paper will discuss how these different hormones are involved and in the way the hormones operate relative to the nature of the primary habitat.

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A review of calcium regulation in vertebrates with special emphasis on the control of hormonal secretion.

Dachs, G.G.
Calcium Regulation in Sub-Mammalian Vertebrates.
A slightly dated but still invaluable book on calcium metabolism in the sub-mammalian vertebrates.
19.2 TRANSEPITHELIAL CALCIUM TRANSPORT IN FISH

Steve F. Perry, Gert Flik & Sjoerd Wendelaar Bonga. Dept. of Biology, University of Ottawa (Canada) & Dept. of Animal Physiology, University of Nijmegen (The Netherlands).

In adult fish, the gill is the predominant site of transepithelial calcium movement although the skin and intestine may be supplementary routes. In early stages of development, the skin may be relatively more important in whole body calcium uptake. In gill and skin, the chloride cell (also termed mitochondria-rich cell or ionocyte) is the cell type responsible for calcium uptake from the water. Thus, inter-specific differences in the rates of calcium uptake in teleost fish can be explained, at least in part, by similar differences in gill chloride cell surface area. Chloride cell requirements, in turn, are "set" by the rate of calcium loss from the internal compartments into the water. Within any given species, modification of the gill chloride cell population can be used as a strategy to alter the rate of transepithelial calcium uptake in accordance with the prevailing water chemistry (e.g. "soft" versus "hard" water).

Transepithelial calcium uptake is a multi-step process beginning with the passive entry of calcium into chloride cells through apical membrane calcium channels and culminating with the active transport of calcium into the blood plasma via a basolateral membrane high-affinity calcium ATPase. In accordance with this model, trans-branchial calcium uptake can be modified by adjustments of apical membrane calcium permeability and basolateral membrane calcium ATPase activity.

19.3 CELLULAR CALCIUM TRANSPORT IN FISH: UNIQUE AND UNIVERSAL MECHANISMS.


Fish take up Ca⁺ via gills and intestine and excrete Ca⁺ via kidneys and intestine. Branchial ionocytes, enterocytes and nephric cells are specialized for transepithelial transport of Ca⁺. Entry of Ca⁺ over the apical membrane, the rate limiting step, is regulated by stannomucin and, at least in enterocyte brush border membrane vesicles, carrier-mediated. Extrusion of Ca⁺ from the cell to the blood is driven by a Na⁺/Ca⁺-ATPase (kidney), a Na⁺/Ca⁺-exchanger (intestine) or both (gills). In tilapia (Oreochromis mossambicus) kept in fresh water (FW), prolactin enhances Ca⁺ uptake and concurrently controls the density of Ca⁺-pumps in the ionocyte plasma membrane. In seawater (SW) fish metabolic clearance and secretion of stanniocalcin is enhanced, in line with requirements for enhanced control over Ca⁺ transport at the apical membrane. In SW tilapia, as compared to FW tilapia, epithelial Ca⁺ influx is comparable in gills, but lower in intestine and kidneys. Accordingly, Ca⁺-ATPase and Na⁺/Ca⁺-exchange activities in FW and SW gills are similar. The extrusion of calcium from the enterocyte is dominated by a Na⁺/Ca⁺-exchanger rather than by a Na⁺/Ca⁺-ATPase, in line with the dependence of intestinal Ca⁺ uptake on the Na⁺ status of the epithelium. Seawater tilapia drink significantly but absorption of Ca⁺ via the intestine is minimized; in parallel, Na⁺/Ca⁺-exchange activity is decreased. Renal cells of seawater fish contain less Ca⁺-ATPase, in line with decreased needs for Ca⁺ reabsorption. Thus, stanniocalcin, unique for fishes among the vertebrates, exerts a universal action in fish Ca⁺-transporting epithelia. Extrusion of Ca⁺ from fish Ca⁺-transporting cells depends on ATP and Na⁺ gradient driven Ca⁺-pumps universal among vertebrates, but unique in their tissue distribution in fish.

REFERENCES:


19.4 EXCHANGES OF CALCIUM WITH THE ENVIRONMENT AND BETWEEN DIFFERENT BODY COMPARTMENTS IN AMPHIBIANS.

Daniel F. Stiffler Calif. State Polytechnic Univ., Pomona 91768

Amphibians possess several epithelia which potentially engage in calcium exchange with the environment. These include skin and gills which transport Ca⁺ from environmental water and the small intestine which takes calcium from food. The kidneys and bladder limit net calcium excretion. Some, endolymphatic sacs and layers in the skin of special species shuttle Ca⁺ to and from extracellular fluid. Early attempts to characterize Ca⁺ exchanges across skin did not clearly establish the nature of the movement of this ion in this tissue. I have found that when Rana pipiens are placed in dilute Ca⁺ solutions they take up Ca⁺ against an electrochemical gradient in a manner that is dependent on external (Ca⁺)⁺ and saturable. Influx and net uptake are enhanced when frogs are acclimated to distilled water and are stimulated by parathyroid hormone. Similar Ca⁺ transport exists in the skin of Xenopus laevis and Ambystoma tigrinum.

REFERENCES:


Early attempt to show Ca transport in frog skin.


Early failure to confirm Ca transport in frog skin.
19.6 MUSCARINAL CONTROL OF CALCIUM REGULATION IN VERTEBRATES

It is known that the plasma calcium level is maintained within a narrow range. The main components in the overall balance of calcium include oral or epithelial intake, renal and bowel excretion, and turnover of storage sites such as bone. All these processes are tightly regulated by hormones. However, there is one component of the balance which has not been fully considered in this overall picture. It is the use of calcium by the body. Calcium is important during specific developmental periods such as growth and reproduction. However, the more important use of calcium is the continuous control of cell viability of almost all cells during the entire life of the organism. How does cellular use of calcium fit into the overall balance picture of our body? Can cellular use of calcium be regulated by the same hormones involved in the other aspects of calcium balance?

Our recent studies with rat and bullfrog suggest that calcium regulating hormones such as parathyroid hormone, estrogen and 1,25(OH) vitamin D can modulate vascular smooth muscle intracellular calcium regulation by its effects on membrane L-type calcium channel (1). The implications of these findings on the overall balance of calcium in the body will be discussed.

REFERENCES:

EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: COMPARATIVE ASPECTS

20.1 EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: INVERTEBRATES.

Although ammonotely is generally thought to be limited to aquatic animals, this talk will describe recent studies which reveal that ammonia is the primary nitrogenous waste in many terrestrial arthropods as well. Excretory mechanisms involve renal, branchial and hindgut epithelia. In gecarcinid land crabs, primary urine is excreted into the branchial chamber and reprocessed by the gills; ammonia is excreted at 3 times the rate of amino nitrogen excretion. Evidence for the importance of the antennal gland in nitrogen excretion was found in ocypode quadrata.

REFERENCES:

Role of amino acids in ammonia sequestration and detoxification in isopods.
20.2
EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: FISHES.
Patrick J. Walsh. U. of Miami, Miami, FL 33149

Patterns of nitrogen metabolism and excretion in fishes are diverse, ranging from nearly exclusive ammonotely (e.g., many teleosts), to nearly exclusive ureotely (e.g., the elasmobranchs), to facultative switching between the two (e.g., the lungfish). However, the paradigm of exclusive ammonotely in aquatic teleosts is being challenged by recent studies: some teleosts are obligately ureotelic (e.g., the Lake Magadi tilapia), and some are facultatively ureotelic, e.g., selected toadfishes. A fully functional hepatic glutamine-urea cycle occurs in the gulf toadfish, Opsanus beta, and this species can switch from ammonotely to nearly complete ureotely within 24 h. This transition is accompanied by an up to six-fold activation of hepatic glutamine synthase (GNS) activity, which traps ammonia nitrogen, shunting it towards urea. Several laboratory treatments can induce this switch (e.g., air-exposure, NaCl exposure, confinement, etc.), with the apparent unifying stimulus being stress. Experiments to date implicate cortisol as one important mediator of the response. Once the fish switches to ureotely, excretion occurs mainly as a single pulse per day from the gills/branchial region, at least in post-absorptive fish. We believe Opsanus beta is often ureotelic in nature, since we find appropriate hepatic GNS activities and plasma cortisol levels in freshly-collected individuals. In addition to studies of the transition to ureotely in Opsanus beta, we have examined other members of the family which are largely ammonotelic. Two traits associated with ureotely in the group are the ability to express mitochondrial GNS activity above a threshold and to increase total GNS activity in response to environmental challenge. Supported by NSF (IBN-9118819).

REFERENCES:


20.3

Adult amphibians are carnivorous and, to achieve nitrogen balance with maintenance levels of food intake, their kidneys must excrete ca. 25 μmoles of nitrogen daily per gram of body mass. Amphibians cannot produce urine that is hypotonic to body fluids, thus the availability of water is critical in determining the mode of excretion of nitrogen-containing compounds. Aquatic forms gain about 0.25 ml of water per gram body mass daily and can thus eliminate excess nitrogen using variable proportions of urea and ammonia in hypo-osmotic urine. Much higher excretion rates (100 μmoles N/g day) can be attained with urine concentrations of 150 mM and U/P ratios of 5. Semi-terrestrial forms do not produce urine while out of water, and urea accumulates in the body fluids. These animals, if feeding, must spend about 10 to 20% of their time in water to eliminate urea as iso-osmotic urine. Terrestrial forms do not produce urine while out of water, and urea is excreted primarily by tubular secretion. Urate, principal nitrogen excretion in urine of some amniotes (e.g., taurine), is often excreted in nature, since we find appropriate hepatic GNS activities and plasma cortisol levels in freshly-collected individuals. In addition to studies of the transition to ureotely in Opsanus beta, we have examined other members of the family which are largely ammonotelic. Two traits associated with ureotely in the group are the ability to express mitochondrial GNS activity above a threshold and to increase total GNS activity in response to environmental challenge. Supported by NSF (IBN-9118819).

REFERENCES:


20.4
EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: REPTILES. William H. Dantzler, Dept. of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724, USA

Major excretory end products of nitrogen metabolism in reptiles are ammonia, urea, and urate. Percent of urinary nitrogen excreted as each varies among orders and species within orders. This variation relates in part to habitat and requirements for conserving water, but metabolic factors, especially acid-base regulation, may also be important. Process of amino acid excretion is also significant. Ammonia excretion, where important, may require tubular secretion as N*=H*. Urea normally undergoes filtration and passive tubular reabsorption. Urate, principal nitrogen excretion in urine of all reptiles except some chelonians, is excreted primarily by tubular secretion. This secretion involves transport into the cells against an electrochemical gradient at the basolateral membrane, probably via secondary active, K*-dependent, countertransport, and movement from the cells to the lumen down an electrochemical gradient via a pathway that does not appear to be mediated. Net secretion is time-dependent and may depend on filtration. Excretion may be related to excretion of inorganic ions and water and to acid-base balance. Some amino acids (e.g., taurine) undergo both net reabsorption and net secretion in renal tubules of opisthodont reptiles. Reabsorption may involve high affinity electrogenic Na*-Cl*-taurine cotransport at luminal membrane and electrochemical 2-3 Na*-1Cl*-1taurine cotransport at basolateral membrane, but secretion steps are not defined.

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Contains a review of types of nitrogenous end products excreted by reptiles and the mechanisms by which such excretion may occur.


Benyajati, S., and S.M. Bay Basolateral taurine transport in reptilian renal cells. Am. J. Physiol. 266 (Renal Fluid Electrolyte Physiol. 35): F439-F449, 1994. This provides information on transport process for taurine at the basolateral membrane of the renal tubules and attempts to integrate this information and information on transport at the brush border membrane into a model for transepithelial transport.
20.5

Excretion of Nitrogen Containing Compounds: Birds.
Eldon J. Braun, Dept. of Physiology, Coll. of Medicine, University of Arizona, Tucson, AZ 85724.

With some exceptions, about 75% of the nitrogen in bird urine is in the chemical form of uric acid or the salts of uric acid. The majority of the remaining nitrogen in the urine is made up of ammonia and its salts. In terms of solubility, the quantity of the various chemical forms of uric acid in the urine greatly exceeds the solubility limits of uric acid and its salts. To prevent precipitation of crystals, a colloidal suspension forms. The suspension is formed by uric acid and a rather large amount (ca. 5 mg/ml) of protein. This suspension exists as small (avg. diameter 3 μm), spherical structures that contain uric acid. This is not a crystalline form of uric acid as is frequently stated in the literature. Furthermore, the uric acid within the spheres is not in a crystalline form, but is chemically bound to a matrix protein. Uric acid in the kidneys enters the colon, and digestive ceca, when the animal possesses ceca. The uric acid and the protein, as part of the urine, are also moved into the GI tract. Within the colon and ceca are large populations of bacteria. A segment of the bacterial matrix protein. Urine from the kidneys enters the lower gastrointestinal tract and is moved by retrograde peristalsis into the colon and digestive ceca, when the animal possesses ceca. The uric acid, and the protein, which may be important for some species. Experimental evidence also indicates that amino acids are transported by the colonic epithelium (which is somewhat unusual for colonic tissue) of birds, suggesting that the protein is also degraded. Supported by NSF.

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PHYLOGENETIC APPROACHES IN COMPARATIVE PHYSIOLOGY

21.1

WHY PHYLOGENIES ARE IMPORTANT TO COMPARATIVE PHYSIOLOGISTS. Raymond D. Huey, Univ. Washington, Seattle, WA 98195

Interspecific comparisons have long been central to explorations of physiological diversity and processes. However, comparative studies have recently been revitalized by the incorporation of an explicit phylogenetic perspective. This perspective not only helps physiologists avoid certain biological and statistical pitfalls, but also provides them with powerful new tools that can be used to address novel issues. For example, a phylogeny provides a crucial guide in the initial selection of species for study as well as a necessary framework for inferring adaptation. Indeed, it allows one to investigate directions of evolutionary change, the number of times a given physiological trait has evolved independently, patterns of correlation between traits, or between traits and the environment, and even rates and sequences of evolution. Thus a phylogenetic perspective encourages a dynamic, historical view of physiological evolution rather than a static one. Moreover, a phylogenetically based analysis of comparative data often leads to different conclusions.

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21.2

What are phylogenies and where do they come from?
Wayne Maddison, Dept. Ecology & Evolutionary Biology, Univ. Arizona, Tucson, AZ 85721

The question of why organisms have the traits they have is often investigated by studying how traits currently function in meeting the challenges faced. Comparative data can show that organisms subject to different challenges in the natural experiment of evolution have responded with different solutions, but if conditions of the natural experiments are ignored (e.g., that replicates are not independent because of phylogeny), the data can be misinterpreted. Passage of genetic information has been constrained to the branches of the phylogenetic tree, and thus the tree has had a profound influence on shaping the similarities and differences of organisms. However, our knowledge of phylogeny varies from group to group. In some, no phylogenetic work has been done in decades; in others, data from numerous sources may convincingly reconstruct the phylogenetic tree. If no well-resolved phylogeny has been worked out for the group of interest, then one might be tempted to continue comparative work without paying attention to phylogeny. We have a natural tendency to scan horizontally across the extant "leaves" of the phylogenetic tree to seek patterns. Such patterns, though, may have little biological meaning. The processes that generated the differences among species operated vertically, along the tree's branches, and we must adjust our view to follow Nature's. If a well-resolved phylogeny is available, then use it to explore the correlates of the evolution of the traits of interest. If one is not available, then one should at least be phylogeny-conscious, by adjusting sampling so as to capture what are likely independent replicates. Any little bit of phylogeny is much better than none at all.

REFERENCES:


21.3
RECONSTRUCTING THE EVOLUTION OF ENDOTHERMY IN FISHES: INSIGHTS FROM MOLECULAR PHYLOGENY. John R. Finnerty and Barbara A. Block. Univ. of Chicago, Chicago, IL 60637.

The suborder Scombroidei is an assemblage of more than 100 marine teleosts that includes tunas, mackerels, marlins, and swordfish. The most noteworthy feature of this group is endothermy. Billfishes (Istiophoridae and Xiphiidae) and the butterfly mackerel (Scombridae) utilize cranial endothermy, warming only the brain and eyes through a thermogenic organ, a region of extracellular muscle specialized for heat production. Tunas warm the cranial compartment, the viscera, and the swimming muscles in a strategy referred to as systemic endothermy. The origin of endothermy in a historical context is central to understanding how and why endothermy evolved in the Scombroidei. Molecular phylogenies based on a mitochondrial gene (cytochrome b) and a nuclear gene (lactate dehydrogenase b) support the same conclusions about the evolution of endothermy in the Scombroidei: that endothermy evolved independently in three scombroid lineages. Phylogenetic analyses of character evolution suggest a link between cranial endothermy and expansion of the thermal niche.

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B.A. Block, J.R. Finnerty, A.F.R. Stewart, J. Kidd
Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny.
Science.

21.4
PHYLOGENY AND THE EVOLUTION OF MUSCLE FUNCTION, MORPHOLOGY, AND BEHAVIOR.
George V. Lauder. Dept. of Ecology and Evolutionary Biology, Univ. of California, Irvine, CA, 92717

One of the central problems in evolutionary physiology is understanding patterns of evolution among different types of physiological traits. Information on the phylogenetic relationships of the species of interest is the basis for reconstructing patterns of physiological evolution, and is essential for interpreting the significance of differences among those species in form or function. Species that are closely related may share functions due to inheritance from a common ancestor and not due to shared present-day environments. In addition, different kinds of physiological traits may show complex patterns of evolutionary change that are not discernible using a non-phylogenetic analysis. For example, in studying physiological changes that underlie evolutionary modifications in behavior, one might quantify the behavior by measuring the pattern of bone movement, then study the musculoskeletal morphology that produces the behavior, analyze motor output from the central nervous system to peripheral muscles, and, finally, study the anatomy and physiology of central nervous circuitry involved in producing the behavior. Interspecific differences in behavior may be generated by changes at one or more of these levels. As one example of an interspecific analysis that considers different types of traits and their evolutionary relationships, I will present an analysis of the evolution of jaw muscle activity patterns, cranial musculoskeletal design, and feeding behavior in six species of aquatic salamanders. The central theme of this presentation is that analysis of different levels of potential physiological change within a phylogenetic framework gives important insights into the evolution of physiological systems.

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Form and function: structural analysis in evolutionary morphology.
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Lauder, G. V.

Lauder, G. V.

21.5
DETECTING CORRELATED EVOLUTION ON PHYLOGENIES: A GENERAL METHOD FOR THE COMPARATIVE ANALYSIS OF DISCRETE CHARACTERS. Mark Pagel, Oxford University, Oxford, UK

I present a new maximum likelihood statistical method for analyzing the relationship between two discrete characters that are measured across a group of hierarchically evolved species or populations. The method assesses whether a pattern of association across the group is evidence for correlated evolutionary change in the two characters. The method takes into account information on the lengths of the branches of a phylogenetic tree, and the relative rates of change of the discrete characters, and tests the hypothesis of correlated evolution with respect to the patterns of evolutionary change in the two characters.

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Detecting correlated evolution on phylogenies: a general method for the analysis of discrete characters.
Proceedings of the Royal Society (B) 255, 1994, 37-45

Harvey, P.H. and Pagel, M.
The Comparative Method in Evolutionary Biology Oxford University Press 1991
21.6 STATISTICAL METHODS FOR TESTING HYPOTHESES ABOUT
THE EVOLUTION OF CONTINUOUS TRAITS.
Theodore Garland, Jr. Department of Zoology, 430 Lincoln Dr.,
University of Wisconsin, Madison, WI 53706-1381

Most characters studied by physiological ecologists and by
comparative physiologists and biochemists show continuous
variation (e.g., maximal or basal rates of O2 consumption, heart
rate, hematocrit, % muscle fiber types, relative organ masses, in
vivo enzyme activities). When species are compared, the typical
approach involves gathering data on the average values for a
series of species (or populations) and then analyzing them with
conventional statistical techniques, such as correlation or
analysis of variance and covariance (e.g., using body mass as a
covariate) in order to detect correlations. Techniques of phylogenetic
analysis have been developed that use separate information on phylogenetic
relationships of species to allow proper analyses. This
assumption is violated because species are hierarchically related. Thus methods
have been developed that separate information on phylogenetic
relationships of species to allow proper analyses. I will outline and
demonstrate two of these, phylogenetically independent contrasts
and computer-simulated null distributions, for which free PC-based
computer programs are available on request from the author.

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Why not to do two-species comparative studies:
limitations on inferring adaptation.
Physiological Zoology 67, 1994, 797-829.

Argues that two-species comparisons are inadequate
logically and statistically; presents worked example of
independent contrasts (J. Felsenstein, Amer. Natur. 125,
1985, 1-15) and shows an application to addressing whether
a single species deviates from an allometric expectation;
discusses statistical power to detect correlations.

Garland, T., Jr., P. H. Harvey, and A. R. Ives.
Procedures for the analysis of comparative data using
phylogenetically independent contrasts.

Applying independent contrasts to real data and
checking branch lengths for statistical adequacy.

Garland, T., Jr., A. W. Dickerman, C. M. Janis, J. A. Jones.
Phylogenetic analysis of covariance by computer simulation.

Outline of the simulated null distribution approach to
testing hypotheses with comparative data and application
to analysis of differences is home range area between
mammalian carnivores and herbivores, yielding surprising
results; discussion and references on treating polymorphisms.

EVENING PLENARY LECTURE

22.0 ENERGY TO BURN: OPTIMIZING FUEL AND OXYGEN PATHWAYS
FOR RUNNING ANIMALS. C. Richard Taylor. Concord Field Station,
Harvard University, Old Causeway Rd., Bedford MA 01730.

Common sense dictates that animals shouldn't be wasteful in building
and maintaining structures they don't use. We would expect they should have
just enough structure to meet functional demands, and we have called this
principle of economic design symmorphosis (Weibel and Taylor, 1981). This
principle results from natural selection and should apply to all levels of
biological organization (Diamond and Hammond, 1992). We have used
the pathway for oxygen in the mammalian respiratory system to test this principle.
Because oxy is a series of interconnected steps on its way from
environmental air to the mitochondria, and we expect structural capacity will
be matched to functional demand at each step. A comparative approach has
provided us with large differences in oxygen flow through the system: more
than 10 fold on a per gram basis between large and small animals; and 3 fold
between animals of the same size adapted for different levels of aerobic
performance (Taylor and Weibel, 1987). We find a good match between
structures and functions at each of the steps, except the lung, which appears to
be held under other design constraints. Recently, we have extended our
studies to include the transport of fats and carbohydrates from the intestine and
stores to the mitochondria. These studies have begun to provide us with new
insights on limitations to fuel and oxygen delivery to mitochondria, and the
effect of diet on these pathways. I will focus on these new findings in my talk.
These studies are a collaborative effort between the laboratories of J.M. Weber
in Ottawa, Hans Hoppeler and Ewald Weibel in Bern, and mine at Harvard.
Supported by grants from the U.S. and Swiss National Science Foundations.

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Ewald R. Weibel and C. Richard Taylor.
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A series of nine papers testing the principle of
symmorphosis at each step in the oxygen pathway.
Maximal O2 flow is varied by using animals of
different size.

C. Richard Taylor, R.H. Karas, E.R. Weibel & H. Hoppeler
Adaptive Variation in the Mammalian Respiratory System
In Relation to Energetic Demand
Respiration Physiology Vol. 69, No. 1, July 1987, 1-127

A series of eight papers testing the principle of
symmorphosis at each step in the oxygen pathway.
Maximal O2 flow is varied by using animals adapted for
different levels of aerobic performance.

Jared Diamond and K. Hammond
The matches, achieved by natural selection, between
biological capacities and their natural loads
Experimental Vol. 48, 551-557

A classic paper testing the matches between structural
capacity and functional demand in the transfer of
substrates across the gut. Functional demand is
varied by cold exposure and larcation.

MORNING PLENARY LECTURE

38.0 WHEN DOES MORPHOLOGY AFFECT PERFORMANCE?
FEEDING, SMELLING, AND SWIMMING WITH HAIRY LITTLE LEGS.
M. A. R. Koehl. University of California, Berkeley, CA 94720-3140

Many animals from different phyla use appendages bearing arrays of
hairs to perform important biological functions such as feeding, gas
exchange, olfaction, and locomotion. Because all these functions
depend on the interaction of the hairs with the surrounding water or air,
we have been studying how fluid motion around and through such
arrays is determined by their morphology and kinematics. Using
mathematical models, microcinematography, and dynamically-scaled
physical models, we found that very small or slowly moving rows of
hairs function as paddles, whereas larger, faster arrays operate like leaky
sieves. We have discovered that different aspects of morphology and
behavior are important in determining the performance of hair-bearing
appendages of different sizes. Our study has revealed conditions under
which there is permission for morphological diversity with little
consequence to performance, versus conditions under which simple
changes in size, speed, or mesh coarseness can lead to novel
mechanisms of operation.

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Fluid flow through hair-bearing appendages: Feeding,
smelling, and swimming at low and intermediate Reynolds

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Hairy little legs. Feeding, smelling and swimming at low

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PHYLOGENETIC APPROACHES IN COMPARATIVE PHYSIOLOGY MONDAY

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PHYLLOGENETIC APPROACHES IN COMPARATIVE PHYSIOLOGY MONDAY

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PHYLLOGENETIC APPROACHES IN COMPARATIVE PHYSIOLOGY MONDAY
31.1 

AFFERENT MODULATION OF VENTILATORY PATTERNS IN LOWER VERTEBRATES. N.J. Smatresk. Department of Biology, University of Texas at Arlington, Texas 76019, U.S.A.

Despite tremendous diversity in respiratory structures, mechanics and media, similar groups of sensory receptors modulate breathing patterns in all vertebrates. There are, however, several interesting trends in the afferent modulation of respiratory patterns that correlate with the transition from water to air breathing. Peripheral chemoreceptors exert dominant control over the relatively regular ventilation of unimodal water breathers. The weak responses of fish to hypoxemia appear to be mediated exclusively by their peripheral (branchial) chemoreceptors. Removal of chemosensitive feedback via denervation decreases ventilation variability and compromises gas exchange, but does not stop ventilatory rhythms in fish. In bimodally breathing fish, air breaths are initiated by peripheral chemosensitive feedback. The transition from single breath to periodic air breathing patterns in anuran and urodele amphibians appears to be developmentally correlated to the appearance of central chemoreceptors. Amphibians are apneic in the absence of adequate central or peripheral chemosensitive feedback, but a variety of single breath and periodic breathing patterns can be produced by altering steady state levels of central and peripheral stimulation. Mechanosensitive feedback via denervation decreases ventilation variability, and may terminate bouts of breathing. Air flow control within bouts is not understood in buccal pump breathers.

REFERENCES:

31.2 


Amphibians use both lungs and gills for gas exchange. The relative role of these gas exchange modes changes with development. Development of the larval form of amphibians is well characterized having 25 stages. We have implemented an in vitro isolated brainstem preparation to study the development of the respiratory pattern generator in amphibians (Rana catesbeiana). Neural output can be recorded from cranial nerves at all stages of development. At intermediate stages of development (XII-XVII), there are neural bursts for both gill and lung ventilation. Intracellular recording from facial motoneurons reveals that the majority receive synaptic input related to both gill and lung rhythm, some related to lung only, while none receive only gill input. Superfusion of antagonists of glycine (strychnine) and GABA (bicuculline) at these intermediate stage of development abolishes gill rhythm but that related to lung persists. Likewise, superfusion of chloride free solution to disable fast-synaptic inhibition abolishes gill bursts but lung rhythm persists albeit with increased burst duration and amplitude. These results suggest that gill rhythm is critically dependent on fast-synaptic inhibition. There is a mechanism that arises early in development to generate lung rhythm that is not dependent on chloride-mediated inhibition and persists throughout development. With development, however, additional features are added that lead to lengthening of the lung burst and an oscillation within the burst. (Supported in part by HL-49486.)

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31.3 


The respiratory control system is functional in the mammalian fetus, capable of generating rhythmic diaphragmatic contraction and responding to peripheral mechanosensitive and chemosensitive stimulation. At birth, there is a rapid transition from placental gas exchange to air breathing. However, considerable maturation of the respiratory control system occurs both peripherally and centrally during postnatal development. Peripheral mechanosensitive and chemosensitive receptors undergo alterations in set-point and myelination of afferent fibers occurs to a large extent postnatally. Membrane properties of both the premotor and motor neuron are altered leading to decreased membrane resistance with increasing age. Respiratory neurons undergo dendritic arborization, synaptic development, alterations in localization of specific neurotransmitters, and changes in receptor subtypes and affinities. These maturation changes result in increasing complexity of the respiratory output both during eupnea and in response to respiratory stimulation by mechanical, chemical or metabolic factors. Furthermore, postnatal development of the respiratory system affords some degree of plasticity to the final configuration of the control system. Thus external factors (e.g. hypoxemia) during the perinatal period may result in temporal changes in development or to permanent alterations in the characteristics of the respiratory control system.

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MODULATION AND PLASTICITY IN VENTILATORY CONTROL. Gordon S. Mitchell, Department of Comparative Biosciences, University of Wisconsin, Madison, WI, 53706.

In recent years, it has become clear that the neural network subserving ventilatory control, like other motor systems, is subject to modulation and/or plasticity. Modulation is a neurochemically induced (permanent) alteration in synaptic strengths or cellular properties, adjusting or even transforming network operation. Neurotransmitters (eg. serotonin) and neuropeptides often act as neuromodulators. An operational definition of plasticity is more difficult, but it may be useful to define plasticity as an alteration in future system performance (ie. ventilatory response) based on experience (eg. stimulus associations, injury, etc.). Plasticity may involve structural and functional alterations. Sensory feedback (eg. proprioception or chemoreception in respiratory motor control) is at the heart of these processes, triggering or guiding mechanisms that lead to changes in structural or functional system characteristics. In many motor systems, preconditions must be satisfied to achieve plasticity (eg. ongoing modulation). Examples of modulation or plasticity in respiratory motor control will be discussed including: 1) sensory 'memories' triggered by peripheral chemoreceptor stimulation (ref 1); 2) short and long term modulation of the exercise ventilatory response (ref 2&3); 3) developmental plasticity of the hypoxic ventilatory response; 4) injury induced plasticity, and 5) classical conditioning. Modulation and plasticity in respiratory motor control may impart flexibility to this important control system, preserving system performance in the face of changing physiological (eg. developmental) or ambient conditions (eg. altitude) (supported by NIH HL 56789)

CHAOS AND BREATHING PATTERNS IN VERTEBRATES AND INVERTEBRATES. Eugene N. Bruce, Center for Biomedical Engineering, Univ. of Kentucky, Lexington, KY 40506

Recent evidence strongly suggests that a significant part of the breath to breath variations in respiratory patterns in some species is not due to randomness but to nonlinear feedback mechanisms which do not attain an equilibrium state. For example in the adult rat variability in breathing pattern can be reduced by vagotomy and induced reversibly by non-varying electrical stimulation of the central vagus nerve (1). This response appears to be linked to neuromechanical reflex control of end-expiratory volume in species with highly compliant chest walls (2), and involves variable deflation related activation of upper airway and chest wall muscles and alteration of timing of the respiratory phases (1,2,3). This temporal variability may represent a "dynamic homeostasis" in which small changes in physiological parameters can potentially alter the dynamic balance significantly and in non-intuitive ways (3). Similar nonlinear (and perhaps chaotic) mechanisms have been invoked to explain certain behaviors in invertebrates, from single neuron firing behaviors to organized reflex activities. (Supported by HL 44889 and HLS0374.)

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ENVIRONMENTAL AND PHYSICAL DETERMINANTS OF MUSCLE PERFORMANCE CAPACITIES

Preparation of birds for migration: is training an important factor? P.J. Butler & C.M. Bishop, School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

It is not clear to what extent intrinsic factors, such as hormonal influences, and increased locomotor activity (training) just prior to migration, effect the increases in mass and the activities of aerobic enzymes that are seen in the pectoral muscle of a number of species of birds (Butler, 1991). In adult tufted ducks, inactivity causes a substantial (45%) reduction in the activity of citrate synthase (CS, Butler & Turner 1988), but supra physiological levels of thyroxine (T4) do not restore CS activity to that seen in free range ducks (Bishop et al 1995).

In a study on barnacle geese, we have determined plasma concentrations of T4, the mass of, and enzyme activities in, the pectoral muscles of barnacle geese from both wild and captive populations, from hatch up to the time of migration. Up to the time of becoming fully fledged (5 weeks old), the development of all the variables measured are the same in both populations. T4 increases during development and may be involved in stimulating aerobic capacity of the muscle prior to fledging. The mass of pectoral muscles is related to body mass in both populations up to the time of migration (approx. 12 weeks) whereas the activity of CS is substantially (54%) greater in the wild population just before migration, even when related to body mass. Thus, flight activity (training) may be involved in the development of the high levels of aerobic capacity of the flight muscles, but does not appear to regulate their mass.

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32.2

OXYGEN SIGNAL TRANSDUCTION AND MUSCLE METABOLIC CONTROL. P.W. Hochachka, Dept. of Zoology, University of B.C., Vancouver.

Few if any tissues sustain the large scale (over 100 fold) changes in ATP turnover rates that are sustained by skeletal muscles. For over 30 years metabolic biochemists have been searching, without success, for metabolic signals which might account for such immense changes in ATP turnover rates in muscles. For many, perhaps most, enzymes in pathways of ATP utilization and of ATP synthesis, an absence of large changes in substrate or product concentrations during up or down regulation of ATP turnover rate leaves effective enzyme concentration as the regulatable parameter. Hypometabolism thus represents masking of catalytic potentials. It must be postulated that an oxygen sensing system is involved in transduction of the oxygen signal and in regulation of ATP turnover. Oxygen sensing and transduction mechanisms are not known; however, two key conditions (near instantaneous transmission to all parts of the cell and near simultaneous activation of multiple enzymes in pathways of ATP turnover) must be satisfied for this kind of control to be workable.

32.3

IS GROWTH RATE A SIGNIFICANT MODULATOR OF MUSCLE METABOLIC CAPACITIES? Dany Pelletier, Helga Guderley, and Jean Denis Dutil

In contrast to the well-known effects of starvation, the impact of growth rate on muscle metabolic capacities has only recently come to light. The indeterminate growth of many fish species makes them ideal for such studies. In cod, Gadus morhua, muscle glycolytic enzyme levels have a much stronger positive correlation with growth rate than mitochondrial enzymes (Pelletier et al. 1993a,b). Season and acclimation temperature do not significantly modify these relationships. However, the correlations between muscle enzyme levels and growth rate differ among species. When growth rates of individual cod are changed, glycolytic enzyme levels in muscle respond rapidly, indicating that high growth rates are not due to high glycolytic capacities. Comparison of muscle glycolytic enzyme levels of laboratory-reared cod (Pelletier et al. 1993a,b) and those of wild cod suggests that wild cod are generally starving or at best growing slightly. As food availability varies seasonally, rapid adjustments of muscle metabolic capacities to food availability (Mendez and Wieser 1993) may facilitate survival.

32.4

Thermally Induced Changes in Fish Oxidative Muscle: The Interplay of Structure and Metabolic Polism. G.D. Sidell, Dept. of Zoology, Univ. of Maine, Orono, ME 04469.

Cold cellular temperature results in an elevated viscosity of muscle sarcoplasm. For example, kinematic viscosity of cytosol from fish muscle increases 

\[ \text{at } T \geq 1.8 \text{to } T^* = 2 - 5 \text{C} \text{ (1).} \]

This highly viscous cellular milieu at cold temperatures may impede intracellular movement or small molecules necessary for both maintenance of metabolic flux and regulation between intracellular compartments. Many fish species that experience cold body temperature seasonally or have evolved at cold temperatures show common characteristics in both structure and metabolism of their skeletal muscle fibers. The fraction of oxidative fiber volume occupied by mitochondria increases from 0.25 \pm 0.02 to 0.45 \pm 0.02 during acclimation of striped bass from 25°C to 5°C and typically ranges from 0.35 to 0.40 in fibers from Antarctic polar species (2). Both whole mitochondrial temperature zone species and polar fish species display preferential reliance upon oxidation of fatty fuels to support aerobic energy metabolism (2,3). Production of mitochondria at cold temperatures elevates the maximal catalytic capacity of mitochondrial enzymes per gram of tissue, helping to compensate for thermal reductions in k_m. Expansion of the mitochondrial cytoplasmic and mitochondrial compartments, compensating for reductions in molecular diffusion coefficients at cold temperature. High mitochondrial volume densities characteristic of cold adapted animals may result in maintenance of a high energetic status of the cellular edentate pool, restricting glycolytic flux and favoring preferential oxidation of non-carbohydrate substrates.

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Dynamic of enzymatic responses to food availability.
32.3
Temperature and locomotion in fish: crossbridges to whole animals
I.A. Johnston, Univ St Andrews, Scotland

Short-nosed sculpin (Myoxocephalus scorpius) around the coast of Scotland experience average temperatures of around 5°C in winter and 13°C in summer. "Fast-starts" used for prey capture have been shown to be modulated following several weeks thermal acclimation. Parameters which can be altered include the maximum forward velocity, acceleration, tail-beat frequency and tail-beat amplitude. Temperature acclimation is also associated with major changes in force generation, maximum contraction speed (Vmax) and in the force-velocity relationship of live muscle fibres. The F-V relationship at 5°C is significantly less curved in muscle fibres than at 15°C-acclimated fish. After normalising the curves for F0 and Vmax it was found that the change in curvature was sufficient to produce a 40% increase in relative power output at 5°C in cold-acclimated fish. However, fibres isolated from cold-acclimated fish show a failure of excitation-contraction coupling at high temperatures. Fast muscle fibres from rostral and caudal myotomes have identical properties in the sculpin (1). The power output of muscle fibres from rostral and caudal myotomes has been measured at various points along the body by the "work-loop" technique, under the constraints operating during prey capture, using in vivo strain and stimulation patterns. The effects of temperature acclimation on size in vivo muscle work and the molecular mechanisms underlying changes in contractile properties will be discussed.

33.1
ONTHEGENESIS OF CARDIOVASCULAR SYSTEMS I: MECHANISMS

CARDIOVASCULAR DEVELOPMENT IN AMPHIBIANS. Werner Burggren,
University of Nevada, Las Vegas, NV. 89154.

Early in development, most free-living amphibian larvae undergo complex changes in cardiovascular anatomy as the major site for gas exchange shifts from external gills to internal gills to lungs. There are equally complex developmental changes in cardiovascular (cv) function. Embryonic/larval heart rate in many taxa rises abruptly soon after heart beat inception, contrary to allometric predictions. At the same time, mean systemic blood pressure in Rana and Xenopus rises progressively with development, from about 1 mmHg at 1 g body mass to about 10 mmHg at 10 g. Early in the development of R. catesbeiana the conus (bulbus) arteriosus plays an important role in generating central arterial pressure, but as development continues the ventricle progressively takes over as the sole blood pump. Cardiac output in Xenopus increases from about .08 mm3/min/g at 2 g body mass to about .7 mm3/min/g at 1 g body mass. Peripheral resistance correspondingly decreases sharply during development, while there are some notable quantizing differences in physiology in larval amphibians compared with embryos of birds, the overall developmental changes in central arterial hemodynamics are qualitatively very similar in both developing amniote larvae and early chick embryos. This suggests that early physiological development of the cv system follows a common plan in vertebrates.

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33.2
MORPHOGENESIS OF THE VERTEBRATE HEART. J.M. Icardo, Dept.
Anatomy & Cell Biology, Univ. of Cantabria, Spain.

Development of the heart starts with the ingression of early mesodermal cells through the primitive streak. Some of these cells are subjected to inductive influences and become committed to heart. As preheart cells migrate forward and form a bilateral crescent, the precardiac mesoderm, which is the first morphological indication of the heart anlage. The precardiac areas migrate toward the embryonic midline and fuse, resulting in formation of a lateral heart tube. Soon, the tubular heart bends and rotates to the right side of the embryo, being thrown into a loop. After loop the heart progressively acquires an adult configuration. Internally, the tubular heart is transformed into a four-chambered organ by development of independent septa that reunite the center of the heart. Concommitant with all these changes, cells of extraembryonic origin (epicardium, blood vessels, neural crest, nerve fibers) reach the heart and form new systems that become integrated in the developing heart. Recruitment patterns and contractile properties of fast muscle fibres (isolated from rostral and caudal myotomes of the short-nosed sculpin) J. Exp. Biol. 105: 251-265, 1993.

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Overview, morphogenesis, late development

Overview, morphogenesis, mechanisms
33.3

GENETIC DISSECTION OF CARDIOVASCULAR DEVELOPMENT IN THE ZEBRAFISH

M. Fishman, Massachusetts General Hospital, MGH Research Ctr.

The power of genetics applied to integrated systems is that it permits the study of the effect of single genes in their normal milieu, that is the intact organism. The zebrafish, Danio rerio, is an organism amenable to genetics and to embryology. The embryo is transparent. The cardiovascular system is assembled over a few days, and is manipulable and resolvable to the level of single cells. By injection of progenitor cells we have identified the region of the embryo that constitutes the "cardiogenic field", and have begun a molecular analysis of this region. In addition, in collaboration with W. Briever and his colleagues, we have pursued a saturation mutagenesis screen with regard to cardiovascular mutants. We have identified more than a hundred which affect critical decisions in cardiovascular morphogenesis. Supported by NIH grant HL 48961.

33.4

VASCULOGENESIS/ANGIOGENESIS DURING DEVELOPMENT.

Robert J. Tomanek. University of Iowa, Iowa City, IA. 52242

Vascular development of the coronary vasculature is related to the increase in wall thickness of the chambers. During early stages of development a lacunar (sinusoidal) system brings blood cells close to cardiac myocytes. In cold-blooded animals this system persists to provide nutrients to part or all of the ventricular wall. In birds and mammals it is replaced by coronary vessels. The time when an effective coronary circulation is established is related to heart size, e.g., early pregnancy in humans, near term in rats. Neovascularization begins when angioblasts derived from the epicardium coalesce to form capillaries (vasculogenesis) a processes followed by vascular sprouting (angiogenesis). Data from our laboratory on rats indicates that a progressive neovascularization occurs transmurally during prenatal development. Fibronectin deposition precedes neovascularization, while collagen IV and laminin appear as tubes form; collagens I and III are not related to tube formation, but the latter is incorporated into the adventitia of arterioles. In vitro experiments indicate that basic fibroblast growth factor stimulates proliferation and migration of undifferentiated cells, while vascular endothelial growth factor facilitates cord and tube formation. Although coronary vasculogenesis/angiogenesis is not yet well understood, recent data suggest that its regulation involves both cellular and extracellular events.

33.6

REGULATION OF VASCULAR DEVELOPMENT

T.H. Adair, J. Hare, and J-P. Montani. Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS 39216

Long-term interrelations between the perfusion capabilities of the vasculature and the metabolic requirements of the tissues often lead to growth of the vasculature to satisfy the tissue needs. The factors that mediate the vessel growth are not well understood, but oxygen has been implicated as a major control element mainly because vessel growth increases during hypoxic conditions and decreases during hyperoxic conditions. The hypoxia-induced increase in vascularity promotes oxygen delivery to the tissues and when the tissues receive adequate amounts of oxygen, the intermediate effectors return to normal levels and vessel growth ceases. Adenosine is thought to be an intermediate effector of the hypoxic stimulus because it is produced in hypoxic tissues and can stimulate growth in some instances. More recent studies indicate that vascular endothelial growth factor (VEGF), a heparin binding, endothelial cell-specific mitogen, is expressed by cells exposed to a hypoxic environment. Adair, T.H., W.J. Gay, and J-P. Montani. Growth regulation of the vascular system: evidence for a metabolic hypothesis. Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 28) 259: R393-R404, 1990.


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Institute of Anatomy and Cell Biology, University of Heidelberg, Germany

Marlies Elger

Functional Morphology of Renal Epithelia in Fish

The nephron begins with a brushborder segment which forms the primary urine by establishing a functional unit and they are lacking in aglomerular fish. In these fish, the reabsorptive segment is present in addition to the reabsorptive portion, which has no antecedents in FW. Clearly, filtration kidneys are widespread and can develop in the absence of a water load; the M-S conclusion does not follow from its premises. However, the conclusion may be correct. Low blood NaCl is an adaption to FW found in vertebrates and invertebrates. It also occurs in SW vertebrates (except hagfish) but not in SW invertebrates which are isoionic with SW. The widespread occurrence of a distal ("diluting") segment also may support a FW habitat for the ancestors of modern vertebrates.

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Functional Morphology of Renal Epithelia in Fish

Marilou Elger

Institute of Anatomy and Cell Biology, University of Heidelberg, Germany

The morphology of renal epithelia of Agnatha, Elasmobranchii, and Actinopterygii is reviewed. The study of glomerular and aglomerular fishes leads to the concept of two basically different strategies in the vertebrate proximal tubule. Reabsorptive segments, which are associated with morphologically distinct features (e.g. apical endocytotic apparatus), are closely related to the ultrafiltration process of the glomerulus. Both glomeruli and reabsorptive segments establish a functional unit and are lacking in aglomerular fish. In these fish, the nephron begins with a brushborder segment which forms the primary urine by secretion of fluid and electrolytes. In many glomerular fish, the secretory proximal segment is present in addition to the reabsorptive portion. Recent studies indicate its involvement in divalent ion regulation.

A diluting segment has been identified on the basis of morphological homology as well as functional features in all classes of fish. The hypothesis that it is generally found in marine fish because of their need to conserve water could be verified only for many teleosts, which represent a rather young branch of evolution. Thus, the existence of a diluting segment is correlated to the type of osmoregulation.

Intercalated cells involved in acid-base transport, were identified by electron microscopy and immunocytochemistry in the cokcting duet in all vertebrate classes, and are missing only in teleosts.

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RENAL FUNCTION IN THE RIVER LAMPREY, Lampetra fluviatilis, IN SEA WATER AND FRESHWATER, INCLUDING OBSERVATIONS ON HORMONE ACTIONS.

J.C. Rankin Odense University, 5230 Odense M, Denmark

Adult river lampreys feed and grow in sea water before their anadromous spawning migration (1). Small quantities of slightly hyperosmotic urine are produced (2) and this has been confirmed by the present studies: urine flow: 5.8 ml/kg/day; 45.1 ± 5.3 mosm/kg hyperosmotic to plasma. Divalent ion concentrations were very high: Mg²⁺ 154 ± 28 and SO₄²⁻ 77 ± 16 mmol/l. In freshwater lampreys produce large volumes of dilute urine to balance the branchial osmotic influx. Following transfer to iso- or hyperosmotic media, urine flow rapidly decreases as a result of reductions in GFR, SNGFR and effective filtration pressure in the glomerulus (3). Vascular and renal actions of vasoactive hormones were therefore investigated. Arginine vasotocin, the sole lamprey neurohypophysial hormone, was always diuretic but angiotensin II, generally thought to have a diuretic effect in lampreys, was pressor and antidiuretic (Broadhead and Rankin, in preparation).

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GLOMERULAR FUNCTION OF THE IN SITU PERFUSED TELEOST KIDNEY: Lessons from Jawless, Cartilaginous and Bony Fish

GLOMERULAR FUNCTION OF THE IN SITU PERFUSED TELEOST KIDNEY: Lessons from Jawless, Cartilaginous and Bony Fish

TUESDAY

34.4

GLOMERULAR FUNCTION OF THE IN SITU PERFUSED TELEOST KIDNEY: Lessons from Jawless, Cartilaginous and Bony Fish

Richard J Balment & Justin M Warne, University of Manchester, M13 9PT, UK.

Teletost fish have a remarkable ability to vary urine output. Various endocrine systems are believed to interact in the control of urine output, primarily by control of glomerular filtration rates (GFR), but investigations in vivo are complicated by the endocrine effects on different tissues. In the in situ perfusion of the trunk of rainbow trout enables controlled haemodynamic and endocrine investigations in vitro, with routine determination of urine output, GFR and the distribution of glomerular states (filtering (F), non-filtering, arterially perfused (NP), and non-arterially perfused (NFP)). The use of this preparation has: (1) demonstrated the effects of variable perfusion-pressure and addition of colloid to the perfusate on glomerular function, (2) provided the first direct evidence of an intrarenal renin-angiotensin system (RAS) in a lower vertebrate, this RAS is activated by low perfusion pressure and the resultant angiotensin II is antidiuretic, (3) enabled investigation of the renal actions of a physiological concentration of arginine vasotocin (10^{-6}M), demonstrating a potent glomerular antidiuretic action by reducing the population of filtering glomeruli to approx 30%, while a similar proportion of NP glomeruli enlarge, (4) demonstrated that 10^{-7}M ecdysone-1 has vasoconstrictor action in the trout and induces a glomerular antidiuresis. Thus, use of the in situ perfused kidney is providing new insights and will ultimately enable integrated studies of endocrine control of teleost glomerular function.

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34.5

Morphological Basis of Renal Function in Elasmobranchs

Hartmut Hentschel

Max-Planck-Institute of Molecular Physiology, Dortmund, Germany

By renal retention of urea, marine chondrichthyan fish can maintain hypertonular body fluids. The renal function in these fish is correlated with a high specialization of the renal architecture. The renal tissue is zonated and exhibits a complex cepellate countercurrent arrangement of portions of each single nephron in the lateral bundles (1). Kidney structure was studied in dogfish, Scyliorhinus caniculus, and skate, Raja erinacea, with light and electron microscopy including cytochemistry and x-ray microprobe analysis of frozen sections (2). The nephron segmentation and its ontogeny was revealed by reconstruction using serial sectioning and computer-assisted 3-D reconstruction (3). The morphological results and the results of physiological experiments were included in a hypothetical scheme of renal function.

In summary, this model implies that: 1. active transepithelial transport of NaCl is performed by the cells of the early distal tubule, which is exclusively located in the bundles. This transport powers a stream of fluid in a central vessel inside the bundle, 2. a negative gradient of urea is produced by countercurrent multiplication of a hairpin loop of neck segment and proximal tubule segment P1 in the bundle, 3. urea leaves the collecting tubule in the direction of the central vessel by countercurrent exchange, hence it is recirculated to the blood circulation.

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34.6

Role of Arginine Vasotocin in fish Osmoregulation.

Richard J Balment & Justin M Warne, University of Manchester, M13 9PT, UK.

A specific radioimmunoassay has been established for the measurement of circulating levels of AVT in teleost fish (1). Plasma AVT concentrations measured in a range of euryhaline and stenohaline fish were between 10^{-8} and 2x10^{-7}M. There were no consistent differences between plasma AVT levels in euryhaline fish (eel, flounder, trout) long-term adapted to fresh water (FW) or sea water (SW). During the initial period of acclimation concentration, while an acute increase in plasma osmolality was associated with increased AVT levels.

In view of the low circulating levels of AVT measured in teleosts, it is evident that of the described dose-dependent effects of AVT on urine production, only the antidiuretic responses are likely to be of physiological significance. In addition to the vascular V1-type receptor for AVT it appears that the teleost nephron also possesses a V2-type receptor, coupled to adenylate cyclase (3). This latter type of receptor was previously considered to be present only in tetrapod kidneys.

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The renin-angiotensin system exists in a variety of teleost fish and appears to be important in the control of blood pressure, blood volume, and renal function (Nishimura, 1987). In mammals, renal renin release is controlled by 1) intrarenal baroreceptors, 2) the macula densa, 3) sympathetic outflow, and 4) various humoral factors (Hackenthal et al., 1990), whereas in teleost fish, renin secretion is primarily regulated by a baroreceptor mechanism that senses the changes in renal arterial pressure. In toadfish (Opsanus tau) kidneys, calcium (Ca) influx via voltage-sensitive calcium channels and a subsequent increase in intracellular Ca²⁺ in renin secretory cells appear to inhibit renin secretion (Nishimura and Maday, 1989), whereas a calmodulin antagonist or removal of extracellular Ca²⁺ increases renin release. In contrast, stimulation of β-adrenoceptors or cAMP production does not evoke the renin release. Furthermore, neither changes in intracellular calcium nor application of calcium ions, prostaglandin E2, or arachidonic acid increased renin secretion, suggesting that Ca²⁺ mediates an inhibitory message for control of renin release underlying the baroreceptor mechanism. Since renin secretory cells are modified vascular smooth muscle cells (VSM cells), we intended to characterize Ca signaling in inadfish VSM. Cytosolic Ca²⁺ signals determined by a fluorescent indicator (fura-2) were increased by K⁺, Bay K 8644, and transiently by Ang II. VSM membrane depolarization contains specific 125I-Ang II binding that was displaced by nonlabeled Ang II and a selective Ang antagonist (losartan). These studies suggest that voltage-gated and hormone-mediated Ca channels and a cellular Ca signaling mechanism are present in teleost VSM.

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ECOLOGICAL PHYSIOLOGY OF ENDANGERED ANIMALS: PHYSIOLOGICAL CONTRIBUTIONS TO THE PRESERVATION OF BIOLOGICAL DIVERSITY

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35.3

PHYSIOLOGICAL ASSESSMENTS OF HABITAT REQUIREMENTS OF A THREATENED FISH. Christina Swanson and Joseph J. Cech, Jr. Dept. of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA 95616.

Populations of delta smelt (Hypomesus transpacificus), a small osmerid endemic to California's Sacramento-San Joaquin estuary, decreased by 90% over the past 20 y. Decreased freshwater inflows and fish entrainment in water diversions in the estuary are among the factors implicated in the fish's decline. We investigated delta smelt's environmental tolerances and their swimming performance behavior in flow regimes like those near diversions. Depending on acclimation temperature and salinity, delta smelt tolerated temperatures from <7°C to >29°C, a range which is within seasonal estuarine conditions. However, temperatures in power plant cooling system diversions may exceed thermal tolerances of the fish. Delta smelt critical swimming velocities averaged 29 cm/sec, which exceeded existing diversion approach velocity regulations, but we observed poor swimming performance at intermediate velocities (6-20 cm/sec). This poor performance may be associated with the transition from intermittent to steady swimming. These results are being used to define delta smelt critical habitat, and develop approach velocity and temperature criteria for diversions in the estuary.

35.4

ACIDIC DEPOSITION AS AN UNLIKELY CAUSE FOR AMPHIBIAN POPULATION DECLINES IN THE SIERRA NEVADA, CALIFORNIA. David F. Bradford, US EPA, P.O. Box 93478, Las Vegas, NV 89193

The Sierra Nevada of California is one of many regions worldwide that has recently experienced dramatic declines in amphibian populations. During the past three decades many populations of at least two species (Rana muscosa and Bufo annunnus) have disappeared in national parks and designated wilderness areas at high elevation, whereas a third widespread species (Pseudacris regilla) has not. Anthropogenic acidic deposition has been proposed as a cause for these disappearances primarily because most surface waters in these areas are exceptionally low in acid neutralizing capacity (ANC), and thus are vulnerable to changes in water chemistry due to acidic deposition. We tested the hypothesis that acidification of habitats has adversely affected amphibian populations, either by itself or in combination with other factors, by eliminating populations from waters most vulnerable to acidification, i.e., low in pH or ANC, or from waters low in ionic strength, a condition that increases the sensitivity of amphibians to low pH. We surveyed 235 potential breeding sites for the above three species at high elevation within 30 randomly selected survey areas, and compared the above chemical parameters between sites containing a species and sites lacking the species. No significant differences were found that were consistent with the hypothesis, and water chemistry did not differ among sites inhabited by the three species. These findings imply that acidic deposition is unlikely to have been a cause of recent amphibian population declines in the Sierra Nevada.

35.5

PHYSIOLOGICAL ECOLOGY OF THREATENED DESERT TORTOISES (Gopherus agassizii), Charles C. Peterson and Kenneth A. Nagy, Univ. of California, Los Angeles, CA 90024-1606.

Measurement of physiological variables in free-ranging individuals of endangered natural populations can help to identify sources of mortality and/or stressors contributing to population declines, and may be used to guide management decisions. Our field studies of the physiological ecology of federally-listed Threatened populations of the desert tortoise (Gopherus [=Xerobates] agassizii) have revealed several features of their biology that are critical in their survival, and which are focal points either for further harm, or for conservation and enhancement efforts by humans. Tortoises are highly dependent on drinking water from summer rainstorms, which allows them to balance long-term water budgets, rid their bodies of accumulated metabolic wastes, store dilute water in their urinary bladders for later resorption, and achieve an energy profit from eating and fermenting dry grasses and annual plants. The spring diet of green annual plants is apparently both osmotically and/or energetically insufficient to balance expenditures, but provides protein nitrogen. Because of high variance in rainfall patterns and concomitant availability of food and water, annual patterns of tortoise energetics and osmoregulation are highly variable among seasons, years, and populations. Western Mojave populations appear to be declining more rapidly than those in the eastern Mojave, which may reflect the rarity and unpredictability of summer rains in the west. During one period of high mortality, physiological measurements and field observations implicated different proximate causes of mortality in two populations. Our findings suggest that conditions for tortoises may be improved by wildlife managers through enhancing the availability of annual wildflowers and drinking water.

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Behavioral evidence that supports the importance of drinking rain water for desert tortoise survival.

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Osmoregulation, water balance, and energetics of subadult tortoises in an eastern Mojave population studied over a full year with use of doubly-labeled water.

The ideals of the conservation-oriented reproductive biologist are similar to those of the conventional livestock reproductive physiologist. Both are interested in salvaging and distributing genetic vigor to ensure preserving species integrity and health. However, the challenges are exponentially greater for the conservation biologist because of the sheer number of species in crisis. The transition of livestock strategies to wildlife will never be simple because of species specificities. Nevertheless, evidence suggests that we are on the edge of a new conservation era that will be both expanded and enhanced by using reproductive technologies including Genetic Resource Banks (GRBs; repositories of sperm, embryos, oocytes, tissue, blood products and DNA). GRBs have profound conservation and management potential, both ex situ (in captive zoo breeding programs) and in situ (in nature). GRBs provide an 'insurance' repository of genes to protect existing wildlife populations from disease epidemics and natural disasters while serving as an invaluable resource for addressing important taxonomy and disease forensic issues. A GRB also provides a means of moving germplasm between wild and captive populations to maximize genetic diversity and species health. Used in concert with artificial insemination, in vitro fertilization and embryo transfer, a GRB could help overcome problems faced by managers including breeding sexually incompatible or geographically disparate individuals. This presentation will (1) describe the use of classical physiological approaches to studying reproductive mechanisms in endangered wildlife species, (2) provide state-of-the-art examples of using assisted reproduction to manage endangered species, and (3) describe the advantages of cryopreservation techniques for conserving bio- and gene diversity.

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ADAPTATIONS TO EXTREME ENVIRONMENTS

36.1

ADAPTATION TO THERMAL NICHES EXTREMES BY A MESOPHILIC BACTERIUM. A.F. Bennett. Ecol & Evolut Biol, Sch Biol Sci, Univ California, Irvine 92717, 0001

Replicated experimental populations of the bacterium Escherichia coli maintained in serial dilution culture for 2,000 generations were used to study the response to selection at both upper (42°C) and lower (20°C) boundaries of their ancestral thermal niche. Ancestral temperature was 33°C. The bacterial adapted more rapidly and extensively to 42°C than to 20°C, judged by improvement in competitive fitness relative to the common ancestor; after 2,000 generations, fitness increased 34% in the former and only 8% in the latter. Adaptation to 42°C was largely temperature-specific, entailing little loss or improvement of fitness at other temperatures. It also did not involve modification of the ancestral limits of the thermal niche. In contrast, adaptation to 20°C entailed significant tradeoffs in fitness. At higher temperatures, fitness relative to the ancestor increased; at 40°C, the average fitness was reduced by almost 20%. Between 20 and 32°C, fitness of this experimental group increased significantly above ancestral values. Both the upper and lower boundaries of the thermal niche were significantly decreased by 1-2°C during adaptation to 20°C. Thus, the pattern of adaptation to extreme environments in this experimental system was asymmetrical with respect to the upper and lower boundaries of the ancestral thermal niche.

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36.2


Antarctic fish are able to swim at -2°C, although maximum speeds are significantly lower than for temperate and tropical species at their normal body temperatures. The contractile mechanisms underlying evolutionary temperature adaptation have been studied in skinned and live fibres isolated from the fast myotomal muscles of fish adapted to a wide range of temperatures. Temperature compensation of muscle power output in polar fish largely involves adaptations in maximum force generation with relatively minor contributions from time dependent contractile properties (1,2). The capacity of the swimming muscles of Antarctic fish for aerobic work is also much lower than for temperate and tropical fish. The mechanisms underlying any temperature-compensation of muscular power output largely involves increasing the numbers and cross-sectional density of muscle mitochondria. The maximum rate of oxygen consumption of isolated red muscle mitochondria from the Antarctic fish (Nototenia corracae) essentially fits on the same rate-temperature curve as mitochondria from a range of temperate, tropical and hot-spring fish (3). The constraints imposed by life in low temperature environments will be discussed.

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**36.3 ADAPTATIONS OF VERTEBRATE RENAL FUNCTION TO EXTREME ENVIRONMENTS.** William H. Dantzler. Dept. of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724, USA

Vertebrate renal adaptations to extreme environments involve primarily regulation of excretion of water and NaCl. In fresh water, vertebrates must excrete excess water and conserve NaCl; in salt water or arid lands, they must conserve water and excrete excess NaCl. This presentation will concentrate on three processes involved in renal regulation of excretion of water in extreme environments: 1) Regulation of initial delivery of water and solutes into lumen of proximal tubule via ultrafiltration of plasma. Regulation of GFR involves both regulation at the individual glomerulus and regulation of the number of glomeruli filtering. 2) Regulation of dilution and concentration of urine. Most vertebrates can dilute initial urinary by reabsorbing filtered solutes without filtered water. Diluting ability is controlled by a variety of factors. 3) Concentrating ability is limited to mammals and birds and depends on concerted action of loops of Henle. In mammals, concentrating ability is most marked in extreme arid environments, but degree still appears to depend on other adaptations. Some birds show limited enhancement of concentrating ability in arid environments, but renal conservation of water in birds is related particularly to regulation of filtration and to excretion of urea. Regulation of water excretion via urea excretion. Regulation of urea excretion may be related to number of filtering nephrons.

**REFERENCES:**


A general review of comparative renal function that considers major aspects of all the topics to be covered in this talk.


A comprehensive review of the most recent data on glomerular filtration in non-mammalian vertebrates.


Includes recent review of urea excretion in reptiles, the class in which such excretion may be most important for water conservation.

**36.4 Control of salt gland function in marine birds**


Review of affere: and afferent control of salt gland function; detailed morphological characterization of underlying cellular aspects.


Review of central nervous actions of ANGII with regard to the control of body fluid homeostasis in birds.

**36.5 ADAPTATION OF THE TILAPIA Oreochromis alcalicus grahami TO ONE OF THE MOST EXTREME AQUATIC ENVIRONMENTS ON EARTH, LAKE MAGADI, KENYA.** Chris M. Wood, Biology, McMaster U., Hamilton, Canada L8S 4K1

Marine birds possess supraorbital salt glands to eliminate excess NaCl from their extracellular fluid (ECF). Increases in ECF toxicity and volume represent the physiological stimuli for salt gland secretion (1). Changes in ECF toxicity are monitored by hypothalamic tonicity receptors which have been characterized electrophysiologically. Alterations in ECFV are monitored by systemic volume receptors utilizing angiotenin II (ANGII) as afferent messenger to the brain. NaCl interaction with specific binding sites in hypothalamic structures lacking the blood-brain barrier, ANGII inhibits salt gland secretion under hypovolemic conditions (2). The salt glands are parasympathetically innervated, with acetylcholine (ACh) eliciting salt gland secretion at elevated organ blood flow due to muscarinic receptor interaction using intracellular calcium and IP3 as second messenger systems (1). Vasoactive intestinal peptide (VIP) is colocalized with ACh in nerve fibers innervating both paraxial and axial tissues. At a potent co-transmitter, VIP stimulates salt gland blood flow and excretion through binding to membrane intrinsic receptors with cAMP as second messenger. Mimicking sympathetic innervation, noradrenalinergic and alph2-agonists cause vasodilatation of the salt gland vasculature with slightly diminished secretion. Neurally released nitric oxide as non-choline+ transmitter at sympathetic neural endings reduces both salt gland blood flow and NaCl excretion. With regard to hormonal control, steroid hormones and prolactin appear to fulfills a role. Avian atrial natriuretic factor transiently stimulates secretion via interaction with high-affinity binding sites distributed throughout the glandular parenchyma (1). In an orchestrated system, the salt glands help to maintain avian body fluid homeostasis, and marine birds would not survive without them.

**REFERENCES:**


Includes recent review of urea excretion in reptiles, the class in which such excretion may be most important for water conservation.
37.1  
DESIGN OF METABOLIC PATHWAYS: DO MUSCLES HAVE ENOUGH, OR TOO MUCH ENZYME?  
Raul K. Suarez, Department of Biological Sciences, University of California, Santa Barbara, CA 93106-9610

Analyses of the factors that determine or constrain the design of physiological systems depend upon meaningful comparisons between capacities and maximum physiological demands or loads. This requires thorough understanding of the properties of the system under consideration. In studies of muscle energy metabolism, the common observation that enzyme catalytic capacities (Vmax values) greatly exceed maximum rates of flux (Jmax) has often led to the conclusion that muscles contain “excess enzyme”. It will be shown that near-equilibrium reactions in glycolysis and the enzymes that catalyze them are such that Vmax values must necessarily exceed Jmax. In contrast, interspecies comparisons of certain nonequilibrium steps reveal that the ratio J/Vmax is low in muscles capable of low maximal rates of glycolysis but approaches (or equals) 1.0 in those that sustain high maximal glycolytic rates. The design of muscle oxidative capacities will be considered. Because oxidative enzymes are localized in mitochondria, and are mostly membrane-bound or membrane-associated, enhancement of oxidative capacities appears to be constrained, at least partly, by the availability of space. Comparisons between species suggest that the flight muscles of insects and humans, which have closely approached (or may have actually reached) the upper limit of mitochondrial volume density and cristae surface density. Estimation of rates of oxygen consumption per mitochondria volume and per unit cristae surface area lead to intriguing biochemical questions concerning mitochondrial architecture as well as physiological questions about the role played by mitochondria in setting the upper limits to VO2max.

37.2  
METABOLIC CEILINGS IN ATHLETES, MOTHERS, AND NERDS. Jared Diamond. Physiology Department, UCLA Medical School, Los Angeles, CA 90024.

What limits the metabolic rate that an animal can sustain over long times while remaining in energy balance by means of food intake? For most species studied to date, maximum observed ratios of sustained to basal metabolic rate (SusMR/BMR) fall in the range 2 - 4, occasionally up to 7. My colleagues and I have studied these metabolic ceilings, and the factors setting them, by pushing animals experimentally to high levels of SusMR. Our experimentally imposed energy demands have included exercise, rapid growth, heat production at low ambient temperature, lactation with artificially enlarged litters for artificially prolonged periods, and combinations of these demands (e.g., lactation at low temperature). It turns out that elevated SusMR involves elevated capacities of energy-producing as well as energy-consuming tissues, whose high maintenance costs contribute to elevated BMR. These considerations help explain why no human athletes can maintain training programs of 20,000 calories per day.

37.3  
Testing the hypothesis of symmorphosis: are linked functional capacities designed economically?  
Erwald R. Weibel, MD, DSc, University of Berne, Switzerland

Symmorphosis predicts that the quantitative design of functional systems is adjusted to match the functional demands imposed on the system and is hence a reflection of economic design. It is based on the hypotheses that (a) structural design determines, to a significant extent, functional capacities of cells and organs, and (b) the capacities of sequential steps in an functional system are adjusted to overall functional capacity. We have tested this hypothesis on the pathway for O2 from lung to mitochondria. By studying variations in aerobic capacity due to body size (allometric variation) and athletic status (adaptive variation) we found that the mitochondria of muscle cells, the muscle capillary network and its erythrocyte content as well as the heart and blood are all coadjusted to VO2max. In contrast, the lung shows a limited excess capacity for O2 uptake, but other studies suggest that this may be related (1) to variations in the environmental O2 and (2) to a limited morphogenetic capacity of this organ. In recently extending these studies to the pathways for substrate utilization (glycolysis and fatty acids) we arrived at the conclusion that the muscle microvasculature is adjusted to the needs for O2 supply, whereas differences in substrate needs are matched by coadjustment of subcellular structures which agrees well with the functional pattern. We conclude that linked functional capacities are to a significant extent designed economically. Apparent exceptions are cases with limited excess capacity which can be interpreted as safety factors for critical steps.

Supported by Swiss National Science Foundation grants.

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Respir Physiol 69:1-127

The concept of symmorphosis: A testable hypothesis of structure-function relationship.  
Proc Natl Acad Sci USA, 88:10357-10361

Outlines the test requirements for the hypothesis of symmorphosis.

Variations in function and design: Testing symmorphosis in the respiratory system.  
Resp Physiol 87:323-348

Summarizes the results on allometric and adaptive variations in the pathway for O2 reporting detailed data and their interpretation.
CAN EVOLUTION OPTIMIZE PHYSIOLOGY? Martin E. Feder, Univ. of Chicago, Chicago, IL 60637.

To what extent are selection and other evolutionary processes sufficient to account for the matching of functional capacity to functional demand, and to what extent do evolutionary mechanisms limit such matching? To encourage discussion, I will emphasize three reasons why the close match of supply and demand may be an unlikely outcome of evolution. (1) Numerous processes (e.g. routine homeovascular, acclimation, training, developmental plasticity) tend to adjust supply to match changes in demand within the lifetime of an individual organism or cell. Such plasticity can mitigate the selection that would otherwise ensue if the phenotype were constant. In some cases, however, the evolved capacity for phenotypic plasticity appears correlated with the variability of functional demands within an organism's lifetime. (2) Natural and/or sexual selection can plausibly account for an approach to matching of capacity to demand in many instances, but a close matching may be more difficult to understand. Problems include: the dubious disadvantages of over-capacity and supra-adequacy, the evolutionary transition from one integrated phenotype to another, and genetic constraints on evolution of optimality. (3) Mechanisms of evolution other than selection may bias outcomes against close matching. Reconciling these considerations with observed matches of supply and demand may be a fruitful area for future study.

Supported by NSF IBN-9408216.

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TUESDAY MORNING PLENARY LECTURE

37.4

EVOLUTIONARY DESIGN OF FUNCTIONAL CAPACITIES: HOW MUCH IS "ENOUGH BUT NOT TOO MUCH"? A-39

REFERENCES:

WEDNESDAY MORNING PLENARY LECTURE

38.0


Insect growth and metamorphosis are regulated by two hormones: ecdysone which causes molting and juvenile hormone (JH) which prevents progression through metamorphosis (1). JH is present throughout larval life and allows molting and continued growth. In the final larval stage ecdysteroids acting in the absence of JH cause a switch to developmental programs to that of the pupa; then a similar switch occurs in the pupa at the onset of the molt to the adult. Changes in both quantity and types of ecdysone receptors and the ecdysteroid-induced transcription factors occur at the time of these switches. Studies on the development of the tobacco hornworm, Manduca sexta, show that JH directly acts on the cells to prevent the ecdysteroid-induced switching (2). We have recently isolated a cDNA encoding a nuclear JH-binding protein for JH (JHBP) that is expressed in the larval epidermis and disappears at the time of the ecdysteroid-induced switch to pupal commitment (3). The sequence of this protein indicates that it has no known DNA-binding motifs and little similarity to other known proteins. Possible roles of the JH-JBP complex in modulating ecdysteroid action include prevention of the switch of the ecdysone receptor isoform and/or of the transcription factor complex induced by ecdysteroids and stabilization of the chromatin structure surrounding active genes. These or other changes at the molecular level would then lead to the ‘status quo’ effects seen at the organismal level.

Supported by NSF and NIH.

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45.0


Biological systems exhibit considerable plasticity in their responses to changing environments, depending on the severity and duration of environmental alteration. This plasticity is evident both phenotypically in individual organisms and genotypically in populations and species during evolutionary adaptation to diverse environments. The acute responses of an individual organism to abrupt environmental change may be modified and sometimes ameliorated by acclimation or acclimatization. Additionally, the physiological phenotype may be permanently affected by environment at some critical ontogenetic phase. Over longer evolutionary time periods, populations may undergo genetic adaptation as their environment changes due to migration or climatic change. Thus, a hierarchy of responses, both phenotypic (acute, acclimatory, and developmental) and genotypic (evolutionary), may be found in biological systems in response to changes in the environment.

There has been much debate as to whether genetically-determined differences among populations should automatically be ascribed to selective pressures, and whether the response of a species to environmental change is a result of natural selection. The debate may be complicated by factors such as pleiotropy or drift and have no associated benefit. It is possible that some of these phenotypic alterations are in fact only correlated properties and are not specifically beneficial in the environments that occasion them. Experimental data will be presented to examine this assumption.
46.1

**Principles of Cold Hardiness in Ectotherms.** Richard S. Lee.

For those ectotherms that can endure subzero temperatures, survival depends on maintaining a supercooled state within their body fluids or tolerating internal ice formation. Freeze intolerant species promote supercooling by removal of efficient ice nucleators, avoidance of inoculative freezing and the accumulation of low molecular weight polyols and sugars and antifreeze proteins. Freeze tolerant species must not only endure the effects of low temperature per se, but cellular dehydration, anoxia and other stresses attendant with internal ice formation. Ice nucleating active microorganisms, recently reported as normal flora in the gut of freeze tolerant insects and frogs, may play a role in inoculating protective freezing at relatively high subzero temperatures. Recent investigations have directly tested the cryoprotective role of glucose in the freeze tolerant wood frog, *Rana sylvatica*; glucose loading allowed frogs to survive previously lethal rates of freezing and low temperature exposure. Another major adaptation of wood frogs is extensive dehydration (of up to 50% or more) during the early hours of ice formation. This water is relocated to the coeloms and lymph sacs where it is sequestered as ice. Organ dehydration functions to limit mechanical damage due to ice formation and by concentrating cryoprotectant in the unfrozen water fraction.

46.2

**Ice Nucleators and Subzero Temperature Tolerance.**

Karl Erik Zachariassen, University of Trondheim, Norway.

Ice nucleating agents (INAs) are substances that cause water to freeze at relatively high subzero temperatures. INAs appear normally to be present in animal cells and the intestine, where they might cause lethal freezing. Some species (insects) seek to avoid lethal freezing in winter by removing these INAs in the fall and thus reducing the supercooling points (SCP's). The removal of INAs also enhances the SCP depressive effect of polyol accumulation. This enhancement may be due to a combination of a colligative effect and a volume effect of polyol hydration.

Insects may also inactivate the intraintestinal or intra-cellular INAs by secreting that freezing is initiated in the hemolymph at a higher temperature, either by inoculation of ice from the exterior or by production of potent INAs in the hemolymph. In addition to this effect, freezing induced at a high temperature may protect by reducing the osmotic stress associated with freezing and by creating a favorable organismal water balance during winter.

The concentration of hemolymph INAs is much higher than that required for ice nucleation at a high temperature. Possible roles of the INA molecules beyond ice nucleation will be discussed.

46.3

**Fish Antifreeze Proteins**

Arthur DeVries

Department of Physiology, University of Illinois, Urbana, Ill. 61801

The survival of marine fishes in freezing seawater (-1.9°C) is linked to the presence of high levels (25mg/ml) of blood borne antifreeze (AF) proteins. The AFs are either glycopeptides (AFP's) or peptides (APP's). They act by adsorbing to ice crystals that inadvertently enter the fish, inhibiting their growth to a temperature slightly below the freezing point of seawater. Fishes of the perennially freezing Antarctic Ocean synthesize AF proteins continuously throughout the year while many northern fishes regulate levels in response to seasonal temperature changes. Although AFs perform a common antifreeze function, they are surprisingly diverse in their structures and sizes both between and within fish species. In the Antarctic cods (notothenioids) and northern true cods the AFs are AFPs composed of the basic repeating glycopeptide unit (Ala-Pro-Ala-Thr), with the disaccharide, galactosyl-N-acetylgalactosamine attached to the Thr's and are present in at least 16 sizes (2,600-33,000Da). Three types of AFPs have been identified: the alanine rich helical type I AFP of flatfishes and sculpins, the cysteine-rich type II AFP of sea raven, smelt and herring and the type III AFP of eel points which are largely random in amino acid composition and in contrast to all others have a compact structure with a molecular weight of 70K. The AFP adsorb to specific ice crystal planes which vary with AF type. Apparent lattice matches have been identified for all the AFPs and the helical peptides. The mechanism of ice growth inhibition results from increases in local surface curvatures which lowers the freezing point and completely inhibits growth of the crystal even though adsorption is at only one interface orientation. This non-colligative mechanism presupposes the presence of ice and indeed Antarctic fishes have endogenous ice throughout much of the year. The secreted fluids (urine, cataract and endolymph) lack AFs and remain supercooled because the tight capillaries are barriers to the passage of blood born "growth inhibiting crystals".

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   Review of various types of antifreeze proteins, their structures and proposed mechanisms of action.

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   Review of various types of antifreeze proteins, their structures and proposed mechanisms of action.

   Antifreeze proteins adsorb to specific ice crystal planes and their molecular alignment is known. Details of the lattice match between the protein and ice plane are presented as well as the inhibition of growth mechanism.
46.4 ANTIFREEZE PROTEINS IN TERRESTRIAL ARTHROPODS AND PLANTS. John G. Duman. Dept. of Biological Sciences, Notre Dame, IN 46556.

Thermal hysteresis proteins (THPs) are produced in winter by many terrestrial arthropods (insects, spiders, mites, conifopilids). In most of these freeze-avoiding species, THPs function as antifreezes by inhibiting intrusive freezing across the cuticle from external ice, and by inhibiting ice nucleators thereby promoting supercooling. A few species of THP-producing arthropods are freeze tolerant. In at least one of these, the centipede Lithobius forficatus, THPs at rather low concentrations (0.2 mg/ml) inhibit damage during freezing and thawing; however, the mechanism is not understood. At this time the most active THP known is from the beetle Dendroaspis caniceps. An interesting feature of the sequence of this 8-kDa protein is that approximately every sixth residue is a cysteine. Our surveys have shown that THPs are very common in the plant kingdom in winter with ~40% of the species surveyed (representing broad phylogenetic diversity) having thermal hysteresis activity. The activity in plants is comparatively low, and it is unlikely that the THPs function as antifreezes in these freeze-tolerant plants. THPs from the bittersweet nightshade, Solanum dulcamara, appear to protect protoplasts from freeze damage, but the mechanism of this cryoprotective action is unknown. The nightshade THP is unusual since it contains ~34 mol% glycine. Thermal hysteresis activity is also present in certain fungi in winter and in certain bacteria after cold acclimation. Once again the function of the THPs in these organisms is not understood.

REFERENCES:

46.5 NATURAL FREEZING SURVIVAL BY AMPHIBIANS AND REPTILES. Kenneth B. Storey. Department of Biology, Carleton University, Ottawa, Canada K1S 5B6.

Studies of the mechanisms of natural freezing survival by frogs and turtles are providing a comprehensive view of the physical and metabolic protection that must be offered to vertebrate organs for effective cryopreservation. Proton magnetic resonance imaging of whole frogs has shown the directional mode of ice propagation through the body and the natural shrinkage of organs as water exits into extra-organ ice masses. During thawing, MRI showed non-uniform melting; core organs (with high cryoprotectant) thawed first, facilitating the early reestablishment of heart beat and blood circulation. Using tissue slices and the techniques of differential solidification and cryomicroscopy, organ-specific features of freezing have been identified in liver, heart, and skeletal muscle of frogs and turtles including the importance of frogs of the natural cryoprotectant in maintaining a critical minimum cell volume in frozen organs and an apparently noncolligative mode of water retention in turtle organs. Studies of the metabolic effects of whole body dehydration on 3 species of frogs have suggested that adaptations supporting freeze tolerance grew out of mechanisms that deal with dehydration resistance in amphibians and that some of the metabolic effects of freeze tolerance, such as cryoprotectant synthesis, are triggered as responses to cellular dehydration. Recent studies of the regulation of cryoprotic glucose synthesis by wood frog liver have shown the regulatory role of protein kinases, the role of α and β adrenergic receptor involvement in triggering and sustaining the glucogenic response, and adaptive changes in membrane glucose transporter proteins. Supported by N.I.H. General Medical Science grant GM 43796.

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46.6 MEMBRANE ALTERATIONS DURING COLD ACCLIMATION AND FREEZING IN PLANTS. Peter L. Steponkus. Cornell University, Ithaca, NY 14853.

Freeze-induced destabilization of cellular membranes is the primary cause of freezing injury. Although all cellular membranes are vulnerable to freeze-induced destabilization, maintenance of the structural integrity of the plasma membrane is a prerequisite for survival because of the central role that it plays during a freeze/thaw cycle (1). Cryomicroscopic studies of isolated protoplasts together with electron microscopy studies of freeze-induced ultrastructural changes have yielded a comprehensive analysis of the phenomenology of freezing injury and the identification of specific 'lesions' in the plasma membrane, which vary depending on the stage of acclimation and the nadir temperature to which the protoplasts are cooled. Until the lesions identified to date (expansion-induced lysis, lamellar-to-H2 phase transitions, and the fracture-jump lesion) all are consequences of freeze-induced dehhydration (2). However, whereas expansion-induced lysis is the result of cellular dehydration and the large osmotic excursions incurred during a freeze/thaw cycle, lamellar-to-H2 phase transitions and the fracture-jump lesion are consequences of the removal of water that is closely associated with cellular membranes. These studies, together with a molecular species analysis of the plasma membrane lipids and procedures to alter the lipid composition of the plasma membrane have provided for mechanistic studies to establish directly that alterations in the lipid composition of the plasma membrane are causally related to its increased cryostability after cold acclimation. Similarly, the extreme difference in the freezing tolerance of winter rye (Secale cereale cv. Puma) and spring oat (Avena sativa cv. Ogle) is associated with genotypic differences in the lipid composition of the plasma membrane (3).

REFERENCES:


47.1 NEUROPEPTIDES AND BEHAVIORAL COORDINATION IN HYDRA STATIC ORGANISMS: Ian D. McFarlane, Diane Hudman, and Kwangmoon Choe

Department of Applied Biology, University of Hull, Hull, HU6 7RX, U.K.

A sea anemone is simply a muscular bag full of sea water. There are several reasons why it is a bag of various shape and indulge in a wealth of complex behaviours. First, there are both longitudinal and circular muscles, arranged antagonistically. These muscles can show local or symmetrical movements, can contract rapidly or slowly, and can show both excitation and inhibition. Secondly, the enclosed sea water is under pressure and muscle relaxation is thus as effective at producing shape changes as muscle contraction. Thirdly, the "simple nervous system" is in reality far more complex than the diffuse networks of molluscs. In the pulmonate snail Lymnaea stagnalis, the integrated networks controlling respiration and heartbeat utilize neuropeptides encoded by the large multivex FMRFamide locus. The tetrapeptide FMRFamide stimulates contractions of longitudinal muscles but not of the coelomocytes. These studies suggest most strongly that there are different receptors for the adipokinetic hormones have proved more readily amenable to structure-activity and receptor studies. A protein-coupled receptor with low density lipoprotein-binding activity, skinksley D.R., et al., 2001, 915-927.

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Tenson, T. et al. An inhibitory G protein-coupled receptor with low density lipoprotein-binding activity suggests a role for lipoproteins in a linked signal... PNAS 91, 1994, 4816-4820

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47.2 MULTIPLE NEUROPEPTIDES, REGULATED BY DIFFERENTIAL RNA PROCESSING, MODULATE CARBOHYDRATE METABOLISM IN LYMNAEA. Nino Santana, Kingsley J.A. Cox, Paul R. Benjaminian and Julian P. Burke, Sains Centre for Neurobiology, The University of Sussex, Brighton BN1 9QG, U.K.

The anemones are anemone with physiologically well-characterized neuronal networks of molluscs provide useful model systems for analysing the roles of neuropeptides in neuronal signalling. In the sea anemones, the integrated networks controlling respiration and heartbeat utilize neuropeptides encoded by the large multivex FMRFamide locus. The tetrapeptide FMRFamide and structurally related peptides are ubiquitously and are widely distributed in the major invertebrate phyla. In Lymnaea, the FMRFamide gene encodes for 13 putative neuropeptides, of which were previously unknown and have now been confirmed by biochemical or biophysical methods. The expansion behaviour of sea anemones may be coordinated by two inhibitory neuropeptides, Antho-KAamide and Antho-RFamide. A high specific activity biologically active probe for these peptides has been developed, and preliminary characterization of achetakinin-binding sites on plasma membranes have elucidated for three of their actions: lipid mobilization in vivo, inhibition of acetate uptake into body fat, and inhibition of insulin RNA synthesis in fat body. These studies suggest most strongly that there are different receptors for the adipokinetic hormones have proved more readily amenable to structure-activity and receptor studies. A protein-coupled receptor with low density lipoprotein-binding activity suggests a role for lipoproteins in a linked signal... PNAS 91, 1994, 4816-4820

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CRAB NEUROPEPTIDES: MULTIFUNCTIONAL AND MULTIHORMONAL ROLES IN PHYSIOLOGICAL INTEGRATION. 
Simon Webster, Sch. Biol Sci, Univ of Wales, Bangor, Gwynedd LL57 2UU, United Kingdom.

From a comparative viewpoint, the (neuro)endocrinology of arthropods has long been of interest. Nevertheless, despite a common ancestry, crustaceans have diverged from the insects with regard to unique mechanisms of hormonal control of growth, reproduction and energy metabolism (Chang, 1993). This theme is exemplified using crab and lobster models by considering the roles of a group of structurally related neuropeptides produced by neurons in the X-organ of the eyestalk, namely the moult-inhibiting hormone (MIH), vitellogenesis-inhibiting hormone (VITH) and crustacean hyperglycaemic hormone (CHH) in the control of growth, reproduction and energy metabolism. Recent research has suggested that the considerable overlap in biological activity of these peptides reflects a complex multihormonal control of individual processes such as a moult and reproduction (Webster, 1991, 1993). Our recent discovery (Weinwright, Webster, Rees) of a novel eyestalk neuropeptide which inhibits the production of methyl farnesoate by the mandibular organs in crabs adds yet another level of complexity to the hormonal control of growth and reproduction: since methyl farnesoate has been implicated in stimulation of oedysesteroid production and vitellogenesis, it seems reasonable to speculate that crustacean moult and vitellogenesis are ultimately negatively regulated a complex interaction of several neuropeptides.

MODULATORY ACTIONS OF PEPTIDES IN THE FEEDING BEHAVIOR OF APLYSIA: CELLULAR MECHANISMS AND FUNCTIONAL IMPLICATIONS. 

When feeding movements of Aplysia are strong and frequent, individual muscles may be unable, in the absence of compensatory mechanisms, to relax fully before their antagonists begin to contract, thus disrupting the coordination of movements required for efficient feeding. Such a disruption of behavior can be eliminated by reducing contraction duration through modification of the relationship between contraction amplitude and relaxation rate, the two parameters that determine the duration of contractions. Since the cholinergic mechanisms of the feeding muscularature contain combinations of neuropeptides that either enhance the size and relaxation rate of muscle contractions, or depress the contraction size without affecting its relaxation, appropriate release of the neuropeptides could shorten the duration of contractions. Measurements of peptide release have demonstrated that the release is appropriate for shortening the duration of contractions when they are strong or frequent. At the cellular level, the enhancement of contraction amplitude is mediated via a cAMP dependent mechanism that involves an enhancement of the Ca current. Peptides act both presynaptically and postsynaptically to reduce the size of muscle contractions through a Ca dependent mechanism. At the presynaptic site, peptides reduce the amount of ACh released from motorneurons, while at the postsynaptic site, peptides depress contraction size by activation of a K current that results in a lesser activation of the Ca current. The enhancement of the release rate and Ca2+ current via cAMP, and appears to involve the phosphorylation of the myosin-associated protein, twitchin, which through its own kinase domain may modulate myofilamentous proteins.

REFERENCES:

47.5

47.6

REGULATION OF THE flip-1 Neuropeptide GENE IN C. elegans

L. Nelson, M. Rosoff, T. Foley, S. Craven, and C. Li. Department of Biology, Boston University, Boston, MA 02215.

Neuropeptides are used as chemical messengers for communication in the nervous system. We have been investigating the regulation of the class of FMRFamide-like neuropeptides by the X-organ of Caenorhabditis elegans. At 30 neurons, or roughly 10% of the nervous system in C. elegans, stain with an anti-FMRFamide antisem. Seven putative FMRFamide-related peptides, all containing an N-terminal FLRFamide, are encoded by two transcripts of the flip-1 gene. Six of the seven predicted FLRFamide-containing peptides have been isolated from whole animal extracts by HPLC purification. Exogenously applied FLRFamide potentiates the effects of acronin in an egg-laying assay.

To examine the transcriptional regulation of the flip-1 gene, we have used lacZ as a reporter gene under the transcriptional control of varying fragments of the flip-1 promoter region for construction of transgenic animals. A promoter element that is sufficient to elicit expression in specific cells in the head of the animal has been mapped to within 382 bp of the start site of transcription. Deletion analysis of this region in transgenic mice is being performed to map this element more precisely.

To analyze further the function of flip-1, we are: 1) performing transposon-insertion mutagenesis to disrupt flip-1; and 2) expressing flip-1 ectopically in all cells. To inactivate flip-1, we are screening by PCR for imprecise excisions of a transposon that has inserted into the upstream promoter region of flip-1. To express flip-1 ectopically, we are generating transgenic animals in which flip-1 is under the transcriptional control of a heat shock promoter.

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Rosoff, M., T. Bürting, and C. Li. The flip-1 neuropeptide is processed into multiple, highly similar FMRFamie-like peptides in Caenorhabditis elegans. Journal of Neurochemistry 1993, 2356-2361

47.4

NEUROHORMONAL PEPTIDES IN INVERTEBRATES: A MODEL APPROACH

A. E. S. Chang

Comparative endocrinology of moulting and reproduction: Insects and crustaceans.


S. G. Webster


S. G. Webster

High affinity binding of putative moult-inhibiting hormone (MIH) and crustacean hyperglycaemic hormone... Proceedings of the Royal Society of London, Series B. 251, 1993, 53-59

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Peptide release
CARDIOVASCULAR DEVELOPMENT IN CRUSTACEANS

Brian R. McMahon and Ka-Hou Chu, Dept. of Biological Sciences, University of Calgary and Biology, Chinese University of Hong Kong.

Circulatory systems of adult crustaceans are extremely diverse ranging from a single contractile vessel in some smaller forms to highly complex systems, functionally equivalent to those of vertebrates, in the larger decapod crustaceans. Very little is known about development of any crustacean circulatory system. Virtually nothing is known of their physiology. This review focuses on development in two circulatory systems; the very simple system of the anostracan Artemia franciscana and the highly advanced system of the prawn Metapenaeus ensis, which approximate the natural anatomical range. In each case morphometrics and functioning of the heart are traced throughout development. In the case of the prawn this is extended to include the developing circulatory system. The development of responses to environmental disturbance and other physiological stimulation will be discussed.

48.2

CARDIOVASCULAR DEVELOPMENT IN FISHES

Peter J. Rombough. Fac of Sci, Dept. of Zoology, Brandon University, Brandon, Manitoba, Canada R7A 6A9

Historically, studies of cardiovascular development in fishes have focused on morphological changes. Detailed descriptions of the timing and pattern of blood vessel formation and resulting shifts in blood flow are readily available for a dozen species (1). Recently, however, attention has begun to shift away from morphology toward the study of cardiovascular function, particularly as it relates to respiratory gas exchange (2). This change in emphasis has been made possible in large part by advances in micro-technology. In recent years techniques have been developed that allow measurement of such basic physiology parameters as ladin pressure, blood pH, blood flow and cardiac output in small organisms. These techniques have yet to be applied to the study of young fish in a systematic fashion but already they have yielded some interesting results (3). In particular, it is now clear that larvae and larvae are not simply small adults. Gas exchange in young fish larvae does not appear to be restricted to any particular site, such as the gills, as it is in older fish. O2 levels are relatively uniform throughout the circulatory system. Indeed, experiments in which larvae were exposed to CO suggest that the circulatory system plays only a minor role in gas transport well into the larval stage. Much remains to be discovered about how the cardiovascular system functions in young fish. For example, we know virtually nothing about when or how the heart comes under neuroendocrine control or the extent to which the peripheral circulation is subject to regulation. Even such basic information as how the cardiovascular system responds to changes in temperature or activity remains to be elucidated.

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48.4

CARDIOVASCULAR DEVELOPMENT IN REPTILES.

Stephen J. Warburton. New Mexico State University, Las Cruces, NM. Perhaps due to limited availability, or to challenging aspects of the embryonic anatomy, the number of studies on reptilian cardiovascular development are limited. Reptilian embryos, however, may provide information on cardiovascular function and evolution which is not available from other vertebrate classes. The wide variety of heart anatomies which exist in reptiles must diverge? Are points of divergence preceded or followed by alterations in cardiovascular function or control? Alligator embryos display a hypoxic bradycardia. If diffusion is the primary limitation to gas exchange in these thick-shelled eggs, there is no advantage in mounting a cardiovascular response. In contrast, in kingkake embryos, hypoxia elicits a prompt and reversible tachycardia. These highly permeable eggs may be both diffusion and perfusion limited, making a cardiovascular response worthwhile. Cardiac responses to hypoxia may develop only in those species whose egg anatomy make this an adaptive response.

We know little physiology of the vascular designs for a placenta (1). We are beginning to understand cardiovascular shunting in adult reptiles, but the potential function of controlled shunting in embryos needs investigation. Placental designs, as well as chorioallantoic designs in oviparous species, provide a multitude of potential shunt patterns. Perhaps the heart designs which exist in adult reptiles are in part due to constraints on embryonic designs. Finally, the mechanisms which bring about cardiovascular redesign at birth or hatching are unknown.
48.3 CARDIOVASCULAR DEVELOPMENT IN BIRDS.
H. Tazawa and P.C.L. Hou, Dept. of Electrical and electronic eng., Muroran Institute of Technology, Muroran, Japan and Dept. of Biology, National Cheng Kung University, Tainan, Taiwan.

Avian embryos develop within a porous eggshell. Under the shell and fibrous shell membranes, the choioallantoic membrane expands to encompass the embryo and contents of the egg with development. The outer surface of the choioallantoic membrane is well vascularized for gas exchange, and the mixed oxygenated and deoxygenated bloods empty into the intra- and extra-cardiac shunts. We review blood circulation in late chick embryos with regard to the cardiovascular system and shunt (1). As blood volume, stroke volume, cardiac output, protein, and blood flow through the choioallantoic gas exchanger and arterial pressure increase with embryonic development. However, developmental changes in heart rate are not correlated with changes in embryonic mass (2). Variability of instantaneous heart rate changes with development and characteristic, transient bradycardia begins to occur during the last stages of development in chicken embryos, which may in part be related to the functional development of autonomic nerves (3). Among oviparous vertebrates, avian incubation is unique in terms of pre-incubation egg storage and the necessity of egg turning for development. However, prolonged pre-incubation storage is detrimental to embryonic development. We show deleterious effects of prolonged pre-incubation storage and lack of turning on the heart rate and oxygen pulse of developing chicken embryos.

48.6 FUNCTIONAL DEVELOPMENT OF THE CARDIOVASCULAR SYSTEM IN MAMMALS. Kent Thornburg, Mark Roller, Georgia Girard and Mark Morton Oregon Health Sci Univ., Dept. Physiol., Pediatrics, Medicine, Portland, OR 97201

The mammalian embryonic heart begins beating in anticipation of a complete circulation perfusing rapidly growing tissues. Even after septation, the heart is not a miniature version of its adult counterpart; immature heart has a unique physiology because of properties of pericardium, myocyte, conduction system, scaffolding and coronary blood supply. The chambers of the fetal heart are anatomically and functionally distinct. The right ventricle has a right shifted pressure-volume relationship compared to the left and thus a larger chamber volume at a given filling pressure. Consequently, right stroke volume exceeds left as both ventricles share similar filling and arterial pressures. The right chamber radius to wall thickness ratio exceeds left, putting the right ventricle at a mechanical disadvantage. Fetal myocytes have fewer myofilaments, mitochondria and less sarcoplasmic reticulum than adult myocytes. Contraction is highly dependent on trans-sarcolemmal Ca2+ transport, thus larger fetal than adult myocyte surface area/volume ratio allows normal contraction in spite of the transmembrane Ca2+ requirement. Coronary flow at maximal conductance is greater in fetal than adult hearts allowing O2 delivery in a hypoxic milieu. Myocytes normally grow by cell division in early embryo life. In rats, myocytes terminally differentiate after birth and growth by hypertrophy. In sheep, this occurs mid-gestation. Pressure loading alters fetal heart growth & function. Left Ventricular Stroke Volume in Fetal Lambs. ThomWarg, KL, Morton, MJ, 95-139, 1994. Preload & afterload affects fetal heart function.

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NEW INSIGHTS INTO THE FUNCTION OF THE VERTEBRATE KIDNEY:
LESSONS FROM JAWLESS, CARTILAGINOUS AND BONY FISH II

49.2 Renal Sodium Cotransport Systems: Diversity and Evolution R.K.H. Kinne, A.J. Morrison Shetler", H, Kipp, Ch. Beven and E. Kinne- Saffran, Max-Planck-Institut für molekulare Physiologie, Dortmund, FRG and Mount Desert Island Biological Laboratory, Salisbury Cove, ME, USA;
Wesleyan University, Middletown, CT, USA; "EXXON Biomedical Sciences, Inc., East Millstone, NJ, USA

Sodium cotransport systems are essential elements in the active renal reabsorption and secretion of a variety of inorganic and organic solutes. Employing flux measurements in isolated brush border membranes and immunocytochemical and cloning strategies the sodium glucose cotransport systems in hagfish (Myxine glutinosa), shark (Squalus acanthias), skate (Raja arinaceas), troutfish (Oxyanus tauri) and flounder (Pseudopleuronectes americanicus) kidneys were investigated. These differences in their phenotype with regard to sodium specificity, affinity to inhibitors and the number of sodium ions translocated, immunoreactivity with specific monoclonal antibodies revealed also differences in apparent molecular weight as did the base sequences (partial or complete) of cloned transport proteins. These data will be discussed with respect to the evolution of the sodium-D-glucose cotransport system and the implication for their structural elements involved in solute transport. This approach of comparative physiology at the molecular level is currently extended to study other transport systems such as the sodium-phosphate cotransporter.

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A.L. Morrison-Shetler, et al. Topography of the sodium-D-glucose cotransporter protein expressed in Xenopus laevis oocytes Biochimica et Biophysica Acta in press
Chloride Secretion by the Rectal Gland: Lessons from the Shark

Franklin H. Epstein, M.D.
Mount Desert Island Biological Laboratory
Salem, ME 04672

The eel-suckling rectal gland of elasmobranchs serves to maintain internal homeostasis by excreting surplus NaCl entering the body from a hypertonic sea. The gland is an easily studied model for active chloride secretion by a variety of epithelial tissues. Lessons from the shark rectal gland include: 1) the mechanism of secondary (Na,K,2Cl) active chloride transport across epithelial membranes; 2) modes of intracellular regulation of ion channels and transporters; 3) neurohormonal stimulation of active chloride transport; and 4) neurohormonal inhibition of chloride transport. Pathways of stimulation and inhibition, newly described in shark rectal gland, may elucidate analogous processes that regulate active chloride transport in mammalian organs.

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Mechanisms of Action of Natriuretic Peptides in the Shark Rectal Gland

Karl J. Karnaky, Jr., Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC.

The shark rectal gland has provided us with one of the most significant model systems for the study of epithelial sodium chloride transport. A recent advance in its study has been afforded by our ability to tissue culture the secretory cells as a flat sheet, amenable to Ussing chamber and short-circuit current analysis. In the last several years great attention has been focused on the regulation of chloride secretion in this tissue by natriuretic peptides. The initial work suggested that atrial natriuretic peptide stimulates chloride secretion indirectly, by causing the release of VIP from nerve endings. More recently it was shown that this hormone can stimulate the cultured rectal gland directly, without involvement of nerves, and that this stimulation involves an increase in intracellular cGMP. A C-type natriuretic peptide has been discovered in shark heart, and this peptide stimulates chloride secretion at 10^{-10} M in cultured rectal glands, suggesting that it is an endogenous secretory hormone. Interestingly, this hormone acts on both the basolateral side and the apical side of the cultured gland. The rectal gland also appears to possess a P-glycoprotein-like transport activity. This latter feature will make the rectal gland an extremely useful model to understand xenobiotic transport, which occurs in the vertebrate proximal tubule.

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Valentich, Karnaky, and Moran Phonoactivation and natriuretic peptide-activated chloride secretion in cultured shark (Squalus acanthias) rectal gland epithelial cells Fish Physiology: Ionoregulation: Cellular and Molecular Approaches (ed. by Wood and Shuttleworth) 14, Academic Press (in press)

RENAL SECRETION IN GLOMERULAR AND AGLOMERULAR FISH


There are some 30 species of agglomerular fish which do not use glomerular filtration and tubular reabsorption as the dominant renal steps in the maintenance of extracellular fluid constancy. Lacking glomeruli they rely on tubular secretion and tubular reabsorption. But there are no qualitative differences between glomerular and agglomerular kidneys (1). Both are approximately isosmotic with plasma; and Na, Cl, Mg, and S are the main electrolytes in both urines. The similarities suggest the primacy of tubular transport, not glomerular filtration, in the formation of urine in glomerular as well as agglomerular marine fish. Indeed, renal proximal tubules isolated from glomerular fish secrete fluid in vitro with concentrations of Na, Cl, Mg, and S similar to those in the urinary bladder (2). Renal proximal tubules isolated from agglomerular fish also secrete fluid in vitro, as expected. Rates of fluid secretions and the composition of fluid secreted by agglomerular proximal tubules are strikingly similar to those measured in glomerular proximal tubules, suggesting similar mechanisms of tubular secretion in agglomerular and glomerular proximal tubules. Central to the epithelial secretion of salt and water in agglomerular and glomerular renal proximal tubules appears to be the secretion of Mg by active transport. Apparently, Mg secreted into the tubule lumen behaves like a Donnan ion that invites the transepithelial redistribution of monovalent ions via a Na- and Cl-permeable shunt pathway in accordance with Donnan equilibrium. The Donnan equilibrium is not attained, however, because the tubule lumen is "open," allowing flow to the urinary bladder. Hence, it is the attempt to reach Donnan equilibrium which is responsible in part for the secretion of Na and Cl into the tubule lumen, for transepithelial voltage, for luminal hyperosmolality, and for the downstream flow of tubular fluid.

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Beyenbach, K.W. Comparative Physiology of the Renal Proximal Tubule Renal Physiology 67: 1985, 222-235

Primary monolayer cultures of winter flounder (Pleuronectes americanus) renal epithelial cells maintained in Ussing chambers have provided a means to characterize the mechanisms and regulation of several transepithelial transport processes. Electrophysiological measurements show that the cell cultures maintain proximal tubule-like properties, i.e., low transepithelial resistance, ionic composition and pH gradients, low transepithelial short-circuit current (2 to 3 pA/cm²), and a relatively high transepithelial potential difference (-60 to -80 mV) due largely to the K⁺ diffusing down its electrochemical gradient.


REFERENCES:

This paper contains details of the original methodology used to culture the flounder renal cells and brief characterizations of transport of several solutes.


Electrophoretic characteristics, including plasma membrane electrical potentials, as well as intracellular signaling processes are reviewed in this paper.


The effects of mild heat shock (i.e., elevation of temperature 5°C for 6 h followed by return to normal incubation temperature) on transepithelial transport are reviewed here.

49.7

SOLUTE TRANSPORT BY FLOUNDER RENAL EPITHELIUM IN PRIMARY CULTURE. J. L. Renfro, Dept. Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269-3042.

Primary monolayer cultures of winter flounder (Pleuronectes americanus) renal epithelial cells maintained in Ussing chambers have provided a means to characterize the mechanisms and regulation of several transepithelial transport processes. Electrophysiological measurements show that the cell cultures maintain proximal tubule-like properties, i.e., low transepithelial resistance, ionic composition and pH gradients, low transepithelial short-circuit current (2 to 3 pA/cm²), and a relatively high transepithelial potential difference (-60 to -80 mV) due largely to the K⁺ diffusing down its electrochemical gradient.


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The effects of mild heat shock (i.e., elevation of temperature 5°C for 6 h followed by return to normal incubation temperature) on transepithelial transport are reviewed here.

49.8

STRUCTURE AND FUNCTION OF NATRIURETIC PEPTIDES AND THEIR RECEPTORS. Yoshio Takei and Shigehisa Hirose, Ocean Technot., i(anagawg227, &pan I r ,- ~~

The importance of the renal system in freshwater fish is often discounted relative to the gills because of the low concentrations of Na⁺ and acid-base equivalents (H⁺) in the urine. In fact, in rainbow trout, the kidney normally transports Na⁺ and Cl⁻ from glomerular filtrate to blood at 3 to 4 times their urine uptake rates at the gills. During exposure to low environmental pH, H⁺ is taken up at the gills, and the kidney serves as the only route for compensating the resulting metabolic acidosis. Urinary Na⁺ output increases greatly and titratable acidity (TA) and urine flow increases cortisot secretion and inhibits urine flow.


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50.1 STRATEGIES OF ANTIOXIDANT DEFENSE
Helmut Sies, Institut für Physiologische Chemie I
Heinrich-Heine-Universität Düsseldorf
Postfach 101007, D-40001-Dusseldorf, Germany

Cellular protection against the deleterious effects of reactive oxidants generated in aerobic metabolism, called oxidative stress (1), is organized at multiple levels (2). Defense strategies include three levels of protection: prevention, interception and repair. Regulation of the antioxidant capacity includes the maintenance of adequate levels of antioxidant and the localization of antioxidant compounds and enzymes. Short-term and long-term adaptation and cell specialization in these functions are new areas of interest. Control over the activity of prooxidant enzymes, such as NADPH oxidase and NO synthases, is crucial.

Synthetic antioxidant mimics biological strategies, e.g. the selenoorganic compound ebselen as a GSH peroxidase mimic (3).

REFERENCES:

50.2 SYSTEMIC ADAPTATIONS IN CRUSTACEANS DURING MODERATE HYPOXIA
Alan C. Taylor Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland

Many aquatic decapod Crustacea are frequently exposed to periods of hypoxia. Animals that regularly experience conditions of reduced oxygen availability often possess behavioural as well as physiological mechanisms that enable them to survive (Taylor & Speer, 1988) During moderate hypoxia, oxygen consumption (Mvo2) is maintained over a range of P02 until a critical P02 (Pc) is reached below which Mvo2 declines sharply and anaerobic metabolism becomes increasingly important. Values for the P02 appear to be correlated with the degree of hypoxia normally experienced, the lowest P02 values are shown by species experiencing more extreme conditions. The respiratory mechanisms by which Mvo2 is maintained during hypoxia have been studied in a number of species. In general, gill ventilation rates increase and heart rate is reduced during hypoxia. Published data on cardiac output, based either on the Fick principle or on dilution techniques, are rather variable. Recent work using a pulsed-Doppler flowmeter has confirmed that cardiac output increases during hypoxia and that redistribution of haemolymph flow through the major arteries may occur (Airriess & McMahon, 1994). Some confusion still exists over the quantitative importance of the haemocyanin (HcY) in oxygen transport of resting animals under normoxic conditions due to the considerable variability in published values for haemocyanin oxygen tensions. Its importance during hypoxia is not in doubt; however, and recent studies of the role of organic compounds such as L-lactate and urate in oxygen transport and ketone bodies and urate as modulators of respiratory pigment function during stress. Physiol. Zool. 63, (1990), 253-287.

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50.3 MULTIPLE FORMS OF ANAEROBOSIS: ENVIRONMENTAL AND SULPHIDE DEPENDENT ANAEROBOSIS
Manfred K. Grieshaber, Institut für Zoologie, Lehrstuhl für Tierphysiologie, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany

Animal energy expenditure is well tuned to the demands of the environment. Of the many physiological and biochemical mechanisms animals use to adapt to their habitat, respiration and oxygen consumption are of particular importance. Oxygen uptake in response to changes in the ambient P02 may be kept constant in a wide range of P02, or may be reduced with decreasing oxygen tensions. At least in the peanut worm Sipunculus nudus the latter pattern of oxygen consumption is not only shown by the intact animal, but also on the cellular level. At a certain ambient partial pressure of oxygen, the critical P02 (Pc), physiological mechanisms are insufficient to augment an aerobic energy metabolism. Below this Pc, anaerobiosis commences. The Pc, and thus the standard metabolic rate, however, is not only influenced by the level of the ambient partial pressure of oxygen, but also by other abiotic ecological factors such as temperature, salinity changes or sulfide. The exposure to sulfide shifts the Pc to higher P02 values and energy provision is reduced due to an sulfide induced anaerobiosis. Energy is mainly provided via the same pathways as during environmental anaerobiosis.

REFERENCES:
OXYGEN DEPENDENCE OF MITOCHONDRIAL ENERGETICS UNDER SEVERE HYPOXIA. MICROTHERMOEALORIMETRY EVALUATION OF EFFICIENCY AND P/O RATIOS

Erich Gnaiger*, Gabriela M€ndez*, Steven C. Handb
*Department of Transplant Surgery, Clinic Interdisciplinary Bioenergetics, University Hospital of Innsbruck, A-6020 Innsbruck, Austria; bDepartment of EPO Biology, University of Colorado, Boulder 80309-0304 USA

Within tissues, mitochondria are protected from high atmospheric O2 levels and high O2 stress, yet hypoxia presents a dangerous state of oxidative energy limitation. The oxygen dependence of mitochondrial respiration remains a controversial topic, despite the importance of low oxygen on animal energetics [1,2]. Disagreement may partly be due to the insensitivity of standard respirometric techniques [3]. We found a surprising similarity of the hyperbolic oxygen dependence in isolated rat liver mitochondria and euryoxic Artemia embryo mitochondria, with P50 values of 0.03-0.06 kPa (~0.3% air saturation). Importantly, when respiration was oxygen limited, the efficiency and P/O ratio of oxidative phosphorylation remained high, in contrast to ADP-limited respiration. These results were obtained by oxygen-injection microcalorimetry and indicate an energetic advantage of metabolic downregulation by low oxygen. Diminished production of reactive oxygen species is a plausible mechanism explaining the high efficiency at low oxygen.

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THE SCHOLANDER LEGACY: FROM SIMULATED DIVING TO MICROCOMPUTERS ON MESOPOUGIC SEALS. P.W.Hochachka, Dept. of Zoology, University of D.C., Vancouver.

For a research legacy as complex as that of Per Scholander's, it is impossible to trace with a single line the path from the past (his work) to the present research scene. However, one message seems to reverberate from all parts of his diverse work; namely, that biological problems should be researched in their natural context. Indeed, the idea of taking the laboratory to the organism, rather than vice versa, could be the singular Scholander legacy applicable to the entire field of comparative biology. Interestingly, in his research on the diving physiology of aquatic air breathing vertebrates - a research area in which he had an enormous impact - Scholander was unable to follow in detail his own research philosophy. Thus although he was able to demonstrate that the 'hard wiring' for the diving response was pretty well universal at least in vertebrates, most of his own work on this in animals was largely restricted to laboratory settings (1). In retrospect, it is perhaps ironic that the 'Scholander' diving response is now often synonymous with the enforced or simulated diving response, since from his basic philosophy, we can be sure that this great scientist/adventurer would have preferred to probe the physiology of animals diving in their natural world. Later comparative physiologists and biochemists have done exactly that, with the help of microcomputers, they have taken up the challenge of the Scholander legacy and quantitatively examined the diving physiology, biochemistry, endocrinology, and behaviour of marine mammals and birds in natural field settings (2,3). The main goals of this paper are (i) to briefly trace the development of the field of diving physiology and (ii) to review its present day status, concentrating mainly on insights arising from work on large seals.

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Harvey Lectures 57 (1962) pp. 95-110

2 Hochachka, P.W.
Balancing conflicting demands of exercise and diving.

3 Fedak, M.A. and D. Thompson
Behavioural and physiological options in diving seals.
9.1
THE EVOLUTION OF METABOLIC RATE IN LARVAL DROSOPHILA
David Berrieon, J. Meabs, and L. Pettides.* Department of Zoology NJ-15,
University of Washington, Seattle WA, 98195, USA, and Department of Genetics
and Biometry, University College London, 4 Stephenson Way, London NW1
2BU, UK.
D. melanogaster evolving in population cages for 9 years at 16.5 or 25 °C
and recently collected from 6 latitudes in Australia show parallel life history
differences. The 16.5 °C flies show faster development and have shorter
larval developmental times than the 23 °C (and the more equatorial) flies,
regardless of developmental temperature. Increased growth rates could be a
result of increased generalized metabolic rates or increased processing capacity
associated with increased feeding and metabolic rates. To help distinguish between these possibilities and
to determine how metabolic rate evolves in response to temperature, we measured
metabolic rates of individual third-instar larvae. All flies were reared in common
gardens at 25 °C for 1 h prior to measurement. The selection lines exhibited significant differences in the scaling of metabolic rate
with mass. The slope of the regression line relating metabolic rate and mass was
steeper in the 16.5 °C flies than in the 25 °C flies regardless of developmental
temperature. With the 16.5 °C flies having lower size-corrected metabolic rates over most of the larval size range. The Australian flies did not
display any differences in metabolic rate. We also investigated the relationship
between larval density and metabolic rate. Flies grown at low densities have
lower size-corrected metabolic rates than flies grown at high density. Overall,
these results suggest that the alteration of metabolic rate can evolve rapidly in
the laboratory and that increased growth rates in the 16.5 °C flies are related to
decreased routine metabolic rate.

9.2
ENERGETICS OF MIGRATING ADULT AMERICAN SHAD (Alosa Sapidissima)
Bill R. Keast and Stephen D. McCormick. Coast Aasounous Fish Research Center,
National Biological Survey, Turners Falls, MA and Department of Biology, University of
Massachusetts, Amherst, MA, 01003.
American shad is the most abundant anadromous fish on the eastern coast of the US.
Due to the increasing use of fish ladders to mitigate unnecessary barriers to migration, it is of
interest to understand the patterns of energy use. Tbelonging with shad (10 males; 10 females) were sampled at each of four Connecticut River sites in 1993
and 1994. 1, 139, 198 and 228 km from the river mouth. Liver, red and white muscle, viscera
and gonad were sampled, weighed and assayed for proximate and biochemical
changes. Hemoglobin increased with migration distance in both sexes while hemocyanin did not,
indicating an enrichment of the hemoglobins within red blood cells rather than a
proliferation of cells. Lactate production increased linearly in liver and in female white muscle.
In the red muscle, GPT was highest in both sexes at the 139 and 198 km sites. 2-Hydroxycetone enzyme A
dehyrogenase (BADH) also showed an activity peak at the intermediate sampling points in
both areas in the liver and white muscle. There was no change in red muscle BADH,
activity. Stored tissue glycogen is quickly metabolized to the lowest reach of the river.
These results highlight the importance that subtle changes in microclimate
may constrain behavior. Under these conditions, Verdins can reduce rates of
metabolic activity and increase metabolic activity. A model soPution containing the
buffers present in the cytosol showed the same temperature dependence as
the muscle. Inhibition of anaerobic metabolism had no effect,
these results suggest that the alteration of metabolic rate can evolve rapidly in
the laboratory and that increased growth rates in the 16.5 °C flies are related to
decreased routine metabolic rate.

9.3
THE TEMPERATURE DEPENDENCE OF INTRACELLULAR pH (pHi)
IN FROG SKELETAL MUSCLE. wanovic. 9.5
9.4
Diet, hibernation, and the antioxidant defenses of ground squirrels. Craig L. Frank. Fordham University, Armonk, N.Y. 10504
Ground squirrels (Peromyscus maniculatus) are herbivores that hibernate during winter. High
dietary levels of the polyunsaturate linoleic acid enhance hibernation, probably because these diets reduce the melting points of stored fats, which makes them more metabolizable at low body temperatures. A biochemical limitation associated with high linoleic acid diet is increased production of toxic lipid peroxides. Linoleic acid is 12 times more likely to produce lipid peroxides than other fatty acids. Nonlinear have several antioxidant enzymes increase as defenses against lipid peroxides. It was thus predicted that for proper hibernation, the levels of these antioxidant enzymes should increase as dietary
linoleic acid content increases and during torpor. These hypotheses were tested in feeding/hibernation experiments with P.
maniculatus. The results of these experiments reveal that in brown adipose tissue, the levels of some antioxidant enzymes increase with dietary
linoleic acid content, and during torpor. This demonstrates that antioxidant enzymes play an
important role in the preparation for hibernation.

9.5
THE EFFECTS OF SOLAR RADIATION AND WIND SPEED ON THE THERMAL BIOLOGY OF A SMALL BIRD. Paul O. Watt and Glenn F.
Waltsberg, Department of Zoology, Arizona State University, Tempe, AZ
AR077-16O1
Small birds, because of their small body mass and high surface area to
volume ratios are tightly coupled to the physical environment. We examined
their effects on solar radiation, and wind speed (0.4 - 5.0 m s⁻¹) and their
interactions on metabolic rates in the Virginia. Shaded leaves are the basis for a small
squirrel monkey (15 °C) to a thermoneutral microclimate by simply remaining
active. The results of these experiments
reveal that in brown adipose tissue, the levels of some antioxidant enzymes increase with dietary
linoleic acid content, and during torpor. This demonstrates that antioxidant enzymes play an
important role in the preparation for hibernation.

9.6
CIRCADIAN RHYTHMS OF HEAT LOSS, HEAT PRODUCTION AND BODY TEMPERATURE IN A CONSTANT ENVIRONMENT IN THE
SQUIRREL MONKEY. Edward L. Robinson* and Charles A. Fuller. University of California, Davis, CA 95616.
Body production (HP) and heat loss (HL) rhythms produce the daily body temperature (BT) rhythm in squirrel monkeys entrained to a light-
dark cycle (LD). While rhythms of BT, HP, and skin temperature are known to persist in constant conditions, we predicted different BT,
HP, and skin temperature rhythms in LD than in DD. While HP and HL, activity
and BT were measured for 7 squirrel monkeys in thermoneutrality (27±0.1 °C), in constant light (LL, 200 lx), with food and
water available ad lib. All variables showed free-running rhythms of similar circadian periods, with activity, BT, HP, and HL rhythms
approximately coupled to each other. Individual monkeys showed 24 to 25.7 hour
periodicity. Like entrained animals, animals maintained a 25.7-hour periodicity. Like entrained animals, animals maintained a 25.7-hour periodicity.
Phased activity in DD. While the activity period (a) was accompanied by elevations in both HP and HL,
changes in HL lagged behind those of HP at the start of a and rest (p)
periods, when BT decreased. Changes in HP and HL
minima were ca. 3 W/kg and maxima between 5.6 and 8 W/kg. Changes in BT, HP, and HL between a and p were less abrupt in DD
than in LD. Activity, changes in HP and HL minima were ca. 3 W/kg and maxima between 5.6 and 8 W/kg. Changes in BT, HP, and HL between a and p were less abrupt in DD
than in LD. Activity, changes in HP and HL minima were ca. 3 W/kg and maxima between 5.6 and 8 W/kg. Changes in BT, HP, and HL between a and p were less abrupt in DD
than in LD. Activity, changes in HP and HL minima were ca. 3 W/kg and maxima between 5.6 and 8 W/kg. Changes in BT, HP, and HL between a and p were less abrupt in DD
than in LD. Activity, changes in HP and HL minima were ca. 3 W/kg and maxima between 5.6 and 8 W/kg. Changes in BT, HP, and HL between a and p were less abrupt in DD
than in LD. Activity,
9.7 METABOLIC ADAPTIVE STRATEGIES OF DRY AND WET ADAPTED ZEBRAFINCHES (TAENIO- 
PUSGUMMUTATUM) ON LOW AND HIGH FAT DIETS. Marco Mayer and Seifried Würtsch. Institute of 
Neurophysiology of the University of Cologne. Robert-Bohr-Str. 39, 50931 Cologne, Germany. 
Zebrafinches are living in arid zones. It is unknown, how low and to what extent water is stored in the food. 
above all the fat content of the food, influences the adaptation to arid environments. It is also not clear, 
whether there is an effect on thermoregulation, muscle activity and energy balance. As zebrafinches 
are well adapted to their xeric environment, one can presume that they are able to produce strong 
metabolism in order to calculate the daily water balance (see table).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO₂ Output</th>
<th>CO₂ Inhale</th>
<th>Hill slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry (Id)</td>
<td>0.02</td>
<td>0.00</td>
<td>0.92</td>
</tr>
<tr>
<td>Wet (Id)</td>
<td>0.02</td>
<td>0.00</td>
<td>0.92</td>
</tr>
<tr>
<td>Dry (lw)</td>
<td>0.02</td>
<td>0.00</td>
<td>0.92</td>
</tr>
<tr>
<td>Wet (lw)</td>
<td>0.02</td>
<td>0.00</td>
<td>0.92</td>
</tr>
</tbody>
</table>

9.9 A MOST EASY FRACTION OF TIDAL VOLUME IS STEP-RELATED IN 
Harvard School of Public Health, Boston, MA 02115; Brown University, Providence, RI 02912.

It has been proposed that a mechanical link between respiration and locomotion aids breathing 
(Bramble & Carrier, Science 219:251). Proposed mechanisms in quadrupeds include acceleration of the "visceral piston" 
(patterns of ad lib. intake, chewing, and gait phenomena) and a spatial relationship of the cutaneous 
bleachers. In a step study, we sought to determine the step-related changes in tidal volume (Vt) in 
2 dogs (-20 kg) treated at a 2-2.8 m/s, respiratory airflow (V̇) around a bias flow was measured with a mask-mounted screen pneumotach. We observed various breathing frequencies, 0.5 to 3 Hz, and coupling ratios, 1:1 to 1:3 (bradypnea). The phase relationship of locomotion and respiration 
was monitored. When steps occurred randomly throughout respiratory cycles, we averaged ventilation volumes (containing V̇ and Vt) over 
many step periods: volume changes related to breathing (V̇) averaged to 
9.6 g, Vt was 2 g. In 2 dogs, Vt ranged between 2 and 16% of V̇. Sow 
this could be an artifact (mask movement), judged from apparent V̇ in phase 
with locomotion during swallowing. We conclude that the mechanical 
effect of locomotor events on V̇ is modest. Other step-related effects not 
detectable by this approach, e.g., intrapulmonary gas mixing, are possible. 
Supported: HL 35420, NSF IBN9306466, Both Israel Anesthesia Found.

9.11 GAS EXCHANGE AND WATER BALANCE IN TERENCEBIONID 
BEETLES: ROLE OF THE SUB-EYTRAL CAVITY. Michael C. 
Lluch, and Maria R.B. Lluch. Department of Biology, University of 
Utah, Salt Lake City, Utah 84112.

In many teraneobionid beetles, the wings covers (elytra) have become 
folded to form a chamber covering the dorsal surface of the abdomen. 
This chamber, the sub-eytral cavity (SEC), arises from the abdominal 
and metasternal spiracles and is thought to reduce water loss associated with gas exchange. By treating the SEC as a "natural" 
respiratory chamber and using flow-through gas analysis, we have 
examined the respiratory and water relations of the xeranom 
teraneobionid Eodora armata. Two respiratory systems were used 
in parallel so that CO₂ emission (V̇CO₂) and water loss (WL) from the 
intubated SEC could be separated from that of the general body 
surface. Unlike many arthropods that release CO₂ in bursts, E. 
armata excretes CO₂ continuously from the SEC. Intermittent 
pressure fluctuations were measured in the SEC and may represent 
ventilation movements. Approximately 85% of the total V̇CO₂ 
occurred from the spiracles opening into the SEC, and water loss from 
the SEC was tightly correlated with CO₂ loss. In contrast, 
water loss was not correlated due to the large cuticular 
and sternal water loss. Surprisingly, external V̇CO₂ from the 
mesothoracic spiracles and CO₂ loss from the SEC were not closely 
coupled.

9.12 VAGAL FEEDBACK AND VENTILATION DURING URETHANE 
"SLEEP" AND HIBERNATION. M.R. Harris* and W.K. 

The role of vagal afferent feedback in the control of breathing pattern 
during different central "arousal states" was assessed in golden-mantled 
ground squirrels. Ventilation was monitored during the wake-like (low 
slow-wave-sleep-like state (III) of isoflurane anesthesia but 2) still 
modulate breathing pattern during situations with reduced respiratory drive.

It has been reported that the Savanna Monitor Lizard (Varanus exanthematicus) has a mechanical limitation in ventilation causing a decreased hypoxic drive in ventilation (Exp. Biol. 47:33-42,1987). This conflicts with the behavior of these animals and their metabolic scope. We obtained metabolic and ventilatory data at rest and during running at the highest velocity that could be sustained for 1 minute using a minimum 2-way non-rebreathing valve (Hans Rudolph 2000) and a pneumotach (Fleisch #00). Flow was integrated for tidal volume. Mixed expired gases were collected in a mylar gas impermeable bag and oxygen and carbon dioxide concentrations were determined. The following data were obtained: (m restrain, n = 4, weight = 1.37±0.25 kg, * p<0.05)

<table>
<thead>
<tr>
<th>Speed (m/s)</th>
<th>VO₂ (ml/min)</th>
<th>VE (ml/min)</th>
<th>tidal volume (ml)</th>
<th>Frequency (BPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.6±0.22</td>
<td>52±0.26</td>
<td>14±5.4</td>
<td>3.8±2.4</td>
</tr>
<tr>
<td>1.65±0.61</td>
<td>7.5±2.6±1.1</td>
<td>502±1±1.0</td>
<td>7.8±2.6</td>
<td>7.4±0.2±7.0</td>
</tr>
</tbody>
</table>

The data show that varanid lizards increase ventilation with exercise by an increase in respiratory frequency compensating for a decrease in tidal volume.

Supported by NIH HL-17731, HL-07712 and NSF IBN-9181936.
GASTROINTESTINAL ADAPTATION OF BURMESE PYTHONS. Stephen M. Secor and Jared Diamond. Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

The mammalian GI tract exhibits modest levels of response to feeding; a consequence of small meal size and high frequency of feeding. Because many snake species consume large prey (> 50% of snake body mass) at long intervals (> 10 days), we predicted that their gut would exhibit a much greater response to feeding. We have found that sit-and-wait foraging Burmese pythons (Python maurus), after consuming a meal, rapidly turn on gastric acid secretion, double the mass of the small intestine, and up-regulate intestinal transport rates of amino acids and glucose by as much as 17-fold. These responses occur together with a tremendous (up to 40-fold) increase in metabolic rate. Hypertrophy and activation of the gut appears partly responsible for this metabolic surge, evident by increases in cell proliferation, microvilli length (5-fold), and glucose metabolism of the intestinal mucosa. The early stage of this response, before any of the meal is absorbed, is possibly fueled by mobilization of fat stores, as suggested by a 60-fold increase in plasma triglycerides. In magnitude, these responses far exceed those of mammals, while their gut possesses cellular and molecular mechanisms similar to those of mammals. Thus, python gut may be a useful model for investigating the mechanisms of digestive adaptation.

Supported by NIH grants GM14772, DK17328, and NRSA F32-DK08878

9.21 BIOCHEMICAL INDICIES OF PHYSIOLOGICAL STATE IN THE MUSSEL MYTILUS CALIFORNIANUS. Jonathan H. Stillman and George N. Somero. Dept. of Zoology, Oregon State University, Corvallis, OR 97331-5454.

We have examined the correlations between whole animal oxygen consumption rates (VO2), and the activities of malate dehydrogenase (MDH), catalase (CAT), and pyruvate kinase (PK) as well as RNA:DNA ratios in the intertidal mussel Mytilus californianus, to generate a predictive index of whole animal in situ physiological state. Animals held in the lab for 4 days had VO2’s of 0.007 mgO2/hr, whereas animals measured immediately upon collection had much higher VO2’s (0.011). Since freshly collected specimens had very full guts, their high VO2’s were likely a result of specific dynamic action. Animals were subsequently held in fed and food-deprived conditions for two months. VO2’s of mussels held for two months without food were significantly lower than those held with food (ANOVA, p<0.0005). Enzyme activities and RNA:DNA ratios were not different in freshly-collected mussels and those held in the lab for 4 days. In gut, mantle and adductor tissues, CS and MDH activities and RNA:DNA ratios decreased, and RNA:DNA ratios increased in fed mussels. Gill CS, gill RNA:DNA ratios, and adductor RNA:DNA ratios were significantly correlated with VO2, but did not explain a large percentage of the variance in VO2 (r2=0.20). Using multiple regression, we created a model for VO2 utilizing a combination of enzyme activities and RNA:DNA ratios which explained much more of the variance in VO2 (r2=0.70) than individual parameters. This model may provide a strong predictive index of field metabolic rate.

9.22 MECHANISM OF WINTER ACCLIMATIZATION: SEASONAL VARIATION IN LIPID METABOLISM OF HOUSE FINCHES. Timothy P. O’Connor. Univ. of Michigan, Ann Arbor, MI, 48104.

House finches (Carpodacus mexicanus) exposed to severe cold have significantly elevated metabolic rates for 4 times longer in the winter than during late spring. Increased thermogenic endurance is a common form of seasonal aclimatization among passerine birds, yet its mechanism is not completely evident. In order to elucidate the physiological mechanism(s) of avian winter acclimatization, seasonal variation in metabolic characteristics, body composition, and lipid mobilization and catabolism were examined in free-living house finches from southeastern Michigan. Although standard metabolic rate did not vary seasonally, cold-induced summits metabolism was 28% greater during winter than late spring. The task facing these birds involves not only attaining elevated metabolic rates, but also fueling increased endurance. Body composition analyses revealed that house finch fat content was significantly greater during winter than late spring. Determinations of plasma levels of free fatty acids suggested that the birds’ ability to mobilize lipids was enhanced during winter. Finally, activities of key metabolic enzymes indicated that catabolic capacity was also greater during winter. These results demonstrate the physiological mechanism of winter acclimatization in house finches involves increased fat stores, enhanced lipid mobilization, and greater catabolic capacity.

9.23 WEANING MAOS DETERMINES HOW STORED FUELS ARE UTILIZED DURING PROLONGED FASTING IN ELEPHANT SEAL PUPS. Lorrie L. Heat and Michael A. Littig. Institute of Marine Sciences, Univ. of Alaska, Fairbanks, AK 99775.

Plasma concentrations of blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), and β-hydroxybutyrate (β-HBA) were measured weekly in northern elephant seal pups to test the hypothesis that body mass at weaning determines how stored body fuels are utilized during prolonged fasting. Thirty pups were separated into three categories depending upon body mass at weaning: low weaning mass pups (LWM, n=7) weighed 6.8 kg at weaning, average weaning mass pups (HWM, n=14) weighed 7.8 to 14.0 kg and high weaning mass pups (HWM, n=14) were > 14.0 kg prior to fasting. All LWM pups departed the rookery after 5 weeks of fasting whereas AW and HWM pups fasted for 9 to 14 weeks. Plasma BUN and NEFA concentrations increased in the LWM pups whereas HWM pups maintained lower plasma levels throughout the fasting period (LWM:5.5±3.0μM; HWM:20.5±8.6μM). Circulating BUN levels were only significantly higher in LWM pups during the first week of fasting (p<0.05), but were always twice as high as in AW and HWM pups. Plasma β-HBA concentrations were lowest in the fasted LWM pups but increased in the fasted HWM pups and HWM pups throughout their fast and peaked at 5 weeks (LWM:43±12.2μM; HWM:3.0±0.9μM). Circulating BUN levels were only significantly higher in LWM pups during the first week of fasting with the exception of lower BUN in HWM pups after 7 weeks of fasting there were no differences seen in fasting metabolic rates in pups over 75 kg weaning mass. Thus, HWM pups may be capable of sustaining a prolonged fasting metabolism longer than average pups. In contrast, pups less than 75 kg at weaning showed higher lipid mobilization than fasted pups and showed the expected peak in ketone bodies (which usually precedes departure from the rookery) earlier in the fast.


Most nitrogen excreted by birds is in the form of urate. Although urates are more expensive to produce than urea or ammonia, they can be excreted with minimal water loss. Hummingbirds have high metabolic rates and are nectarivores, so their rates of water flux are very high. Urate excretion is an efficient process because uric acid is more water soluble than either urea or ammonia. The dietary amino acid precursor of uric acid is the amino acid, aspartate. Since aspartate is not found in the diet of hummingbirds, the dietary source of aspartate is unknown. However, our recent study of nitrogen metabolism in hummingbirds (Calypte anna) with either high or low rates of water loss during fasting suggests that the rate of urine excretion was low, more than 55% of urinary nitrogen was excreted as ammonia, but less than 40% as urea. This apparent switch from urate to ammonia as the urinary nitrogen excretion product results in a lower water usage and a lower overall dietary nitrogen requirement. This may be a consequence of the need to minimize urinary electrolyte loss. NH₄⁺ can substitute for K⁺ in Na⁺/K⁺+Cl⁻ transport, which plays a major role in electrolyte balance. This would decouple Na⁺ reclamation from K⁺ transport and provide a means of minimizing both sodium and potassium excretion.

Supported by NSF Grants IBN 9210007 and IBN 9307074 to C.A.B.

9.25 PHYSIOLOGICAL EFFECTS OF NEARSHORE NUTRIENT AVAILABILITY ON INTERTIDAL MUSSELS AND THEIR PREDATORS. Elizabeth P. Dahloff, Bruce A. Menge and George N. Somero. Oregon State University, Corvallis, OR. 97331-2914.

We examined seasonal and microhabitat variability in the nutritional status of two species of rock-intertidal mussels (Mytilus californianus and M. trossulus) and their predators (Pisaster ochraceus and Nucella lamellosa) at two sites along the Oregon coast that differ in nearshore food availability. The ratio of RNA to DNA in adductor muscle (mussels) or foot (snail and whelk), an indirect measure of protein synthetic capacity, was used as an indicator of nutritional status. M. californianus living at the site with higher food availability (Strawberry Hill: SH) had significantly higher RNA:DNA ratios than conspecifics at a site with lower food availability (Boiler Bay: BB), suggesting a greater potential for protein synthesis for mussels living at SH. This difference was maintained throughout the year and was especially pronounced following periods of upwelling. At both BB and SH, mussels and whelks exposed to heavy wave action had higher RNA:DNA ratios than conspecifics living in more sheltered microhabitats, although the differences were not significant in all cases. This pattern was observed consistently throughout the year, suggesting that both nearshore food availability and feeding time may directly affect the capacity for new protein synthesis, and therefore growth, in these organisms.
9.27
THE MECHANICS AND ENERGETICS OF HUMAN HAND-RUNNING. James W. Glassan* and Thomas A. McMahon, Harvard University, Cambridge, MA 02138
To determine how non-locomotor limbs (arms) differ from locomotor limbs (legs), we trained human subjects to run on their hands while supporting a fraction of their body weight. We find that the limb stiffness of the human arm increases by 135% over less than a four-fold range in peak vertical force. In contrast, human legs and a variety of other mammals' locomotor limbs maintain a constant stiffness, regardless of loading, for normal running. In addition, we explored the energetics of locomotion in hand running. The metabolic cost of force generation (joules / Newton) is invariant with speed, as is found in normal legged locomotion. However, our results show that the metabolic cost of force generation while running on human arms is four to five times greater than the cost of force generation for the locomotor limbs of running quadrupeds.

Key words: biomechanics, locomotion, pectoral girdle, shoulder, springs, arms, limb design

9.29
PERFORMANCE OF THE TRUNK MUSCLES IN HYLIDS DURING MATING CALLS. M. Sengbahkara* and R.L. Marsh, Biology, Northeastern University, Boston, MA 02115
Muscle performance has often been quantified using in vitro measurements under simple loading conditions. However, muscles often operate in vivo with varying velocity and force. To design in vitro experiments that will reliably determine the limits of mechanical performance, a detailed understanding of the in vivo cycles of shortening and activation is required. We used high-speed video and electromyography (EMG) to measure the length change pattern and excitation-contraction activity of the trunk muscles (external and internal obliques) in Hyla versicolor (gray tree frog). These muscles undergo high frequency cyclical contractions to perform work, during level running. Muscle force and fascicle length were measured in the medial tensor fasciae latae muscle, on the bony tendon, and the muscle could perform if operating at peak power output. This supports the hypothesis that muscles are used primarily to provide force, rather than perform work, during level running. Supported by NIH grants R01AR418140 to C.K. Taylor, AK39138 to R.L. Marsh and NSF graduate fellowship to T.J. Roberts.

9.35
RESPIRATORY QUOTIENT (RQ) IN DROSOPHILA MELANOGASTER DURING STARVATION AND DESICCATION. Minos Djewand* and Timothy J. Bradley, Department of Ecology and Evolutionary Biology, University of California Irvine, Irvine, Ca. 92717
Selection for postponed senescence in D. melanogaster results both in the extension of life and increased resistance to certain stress characters such as desiccation and starvation. Further selection on these stress characters has produced flies with increased stress resistance beyond that of flies selected for postponed aging. Studies of starvation and desiccation resistance suggest that there is a correlation between the lipid content and starvation resistance on the one hand and glycogen content and desiccation resistance on the other. Indirect evidence suggests that lipid and glycogen are used preferentially as metabolic fuels during starvation and desiccation, respectively. Measurement of RQ allows a direct determination of the substrate being metabolized. Using a Sable respirometry system with simultaneous measurement of O2 metabolism and CO2 release we have measured the respiratory quotient of flies during starvation and desiccation. Flies in groups of 200 were provided with food (controls), with non-nutrient agar (starved), or with no food or water (desiccated), and RQ was monitored for 12 hours. The metabolic responses of control lines not selected for stress resistance were compared to those lines which had undergone many generations of selection for starvation and desiccation resistance. Supported by grant US-PHS AG09970.

9.36
EFFECTS OF BODY SIZE AND THERMAL ACCLIMATION ON PARVALBUMIN CONCENTRATION IN WHITE MUSCLE OF STRIPED BASS. Kenneth J. Rodrick and Bruce D. Sidell, Dept. of Biological Sciences, Idaho State Univ., Pocatello, ID 83209 and Dept. of Marine Sci., Univ. of Maine, Orono, ME 04469.
Parvalbumin from anadromous white skeletal muscle of striped bass (Morone saxatilis) was characterized and hypotheses tested that parvalbumin concentration varies with body size and thermal acclimation. Two parvalbumins (isotypes (molecular weights 8.8 and 10.3 kDa, with pI values 4.4 and 4.9, respectively) were identified from whole muscle extracts by SDS-PAGE, immunoblotting with a monoclonal antibody specific for parvalbumin, and 2-D PAGE of heat-stressed samples. Total concentrations of parvalbumins were exceptionally high, ranging from 9.5 mg to 0.5 mg or 0.55 to 1.32 millimoles per kg wet weight of tissue. There was a significant inverse relationship between body size and total parvalbumin titer in white muscle (r = -0.89, P <0.001), with small bass (<500 g) having two-fold as much parvalbumin as larger fish (>4 kg). However, only concentration of the 8.7 kDa isotype varied between animals of different size. Because parvalbumin can bind two calcium ions with high affinity, the higher titer of parvalbumin in fish of smaller size may contribute to higher tail-beat frequency and faster rate of muscle relaxation than in larger animals. Lower titer of parvalbumin also may prolong the active state of muscle contraction in large striped bass and promote higher muscle force production than in small animals. In contrast to the effects of body size on parvalbumin content, thermal acclimation at cold temperature (9°C) for 8-10 weeks did not change the concentration of parvalbumin in white muscle. (Supported by NSF grants DDE-8411269 to R.D.S. and Marine FOPSC/579 01730 and Dept. of Biology, Northeastern University, Boston, MA 02115.
How much of the work of running can tendons do? Because running on level ground involves negligible net work on the environment, an ideal animal should store and release all of the energy in a step, allowing muscles to generate force economically. To determine whether real animals approach this ideal, we have compared the amount of energy stored and released in a tendon to the work done in the muscle during running. Muscle force and fascicle length were measured in the adductor longus and gastrocnemius of wild turkeys (Meleagris gallopavo) running from 1.5 to 3.5 m/s. Muscle force was measured with two strain gauges mounted on the bony tendon, and muscle length was measured with sonomicrometer crystals mounted along a muscle fascicle. Tendon stiffness and peak isometric force were measured in situ. Muscle force increased linearly with speed and reached values as great as 130N in a muscle with a peak isometric force of 220N. The muscle performed both positive and negative work during a step. The maximum tendon energy recovered equaled the positive work done by the muscle, but both of these were small (<1.5kg.m). While muscle forces were as great as 90% of peak isometric force, the most work done in a step was less than 5% of the work the muscle could perform if operating at peak power output. This supports the hypothesis that muscles are used primarily to provide force, rather than perform work, during level running. Supported by NIH grants R01AR418140 to C.K. Taylor, AK39138 to R.L. Marsh and NSF graduate fellowship to T.J. Roberts.

9.38
THE WORK OF RUNNING: DO TENDONS PULL THEIR WEIGHT? T.J. Roberts, B. L. Marsh, C. J. Buehham, P. G. Weyand and C. P. Taylor, CFS, Harvard University, Old Causway Rd, Bedford, MA, 01730 and Dept. of Biology, Northeastern University, Boston, MA 02115.
A mismatch between the contractile capacity of a calling and note repetition rate during call production in grey tree frogs. M. Sarbadhikary* and R.L. Marsh, Biology, Northeastern University, Boston, MA 02115.
A note repetition rate (NRR) of 28.3 notes per second (rips) at 15°C, 42.5 nps at 20°C, and 56.8 rips at 25°C whereas H. versicolor* (48 chromosomes) has a NRR of 14.6 nps at 15°C, 20.9 nps at 20°C, and 27.3 rips at 25°C. The call consists of 14-15 cycles with a cycle frequency of 15-25 Hz. A maximum tendon energy recovered equaled the positive work done by the muscle, but both of these were small (<1.5kg.m). While muscle forces were as great as 90% of peak isometric force, the most work done in a step was less than 5% of the work the muscle could perform if operating at peak power output. This supports the hypothesis that muscles are used primarily to provide force, rather than perform work, during level running. Supported by NIH grants R01AR418140 to C.K. Taylor, AK39138 to R.L. Marsh and NSF graduate fellowship to T.J. Roberts.
9.31


Sound-producing muscles operate at frequencies 10-100 times greater than those of typical skeletal muscles. In the toadfish swimbladder muscle (TSM) and rattlesnake tail-shaker muscle (RTSM), we have compared the mechanical properties of super-fast muscles with those of traditional slow muscles.

9.32


The effects of epinephrine (Epi), atrial natriuretic peptide (ANP), and angiotensin II (ANG II) on vascular capacitance were investigated in trout (Salmo gairdneri). Changes in mean arterial blood pressure (MAP), heart rate (HR), and tail venous blood flow were measured.

9.33


The effects of arginine vasotocin (AVT) on the isolated gar hepatic veins were examined. AVT produced dose-dependent, long-lived (> 30 min) and large (> 10X greater) relaxation of the isolated gar hepatic veins.

9.34

EXOGOOGENOUSLY APPLIED AND ENDOGENOUSLY DERIVED ATRIAL NATRIURETIC PEPTIDE MEDIATE VASCULAR CAPACITANCE IN THE YELLOWFIN TUNA (Thunnus albacares): CARDIOVASCULAR RESPONSES, PLASMA NATRIURETIC PEPTIDE CONCENTRATION AND DISTRIBUTION OF IMMUNOREACTIVE GRANULES. John E. Kent, Kady L. Cousins, Antonio F. Parmigiani, Richard W. Huestis and Xang X. Ong. Hopkins Marine Station, Stanford University, Pacific Grove, California USA 93950, and Indiana University School of Medicine, University of Notre Dame, Notre Dame, Indiana USA 46556.

Randomized catheter-mediated infusion of rat atrial natriuretic peptide (ANP; final concentrations of 0.0, 0.01, 0.10, 1.00 and 10.00 μg kg body weight^1) into the ventral aorta (VA) of spanning-blocked yellowfin tuna increased VA pulse pressure and mean VA pressure in a concentration-dependent manner. These effects were mirrored by concentration-dependent increases in mean heart rate. In a separate study, 2 hr infusion of controlled (thriboin, C-ANP) which, in mammals, inhibited removal of ANP from circulation, produced similar cardiovascular responses. Radioactivity of blood samples taken over the course of this study demonstrated a significant increase in endogenous natriuretic peptide levels with inhibitor infusion. Peptide levels returned to pre-infusion levels with cessation of blocker infusion. Finally, immunohistochemical analysis of sample heart revealed the presence of immunoreactive particles in the atrium with few such particles being found in ventricular tissue. This distribution pattern is similar to that found in other teleosts and, taken together, these results suggest that natriuretic peptides play a role in the regulation of blood pressure in tunas as they do in other vertebrates.
REGIONAL CEREBRAL BLOOD FLOW DURING SIMULATED DIVING IN THE RAT. Onken P., Oldenburger and Nigel H. West, University of Saskatchewan, Saskatoon, Canada, STN (DW).

The objective of this study was to examine the cerebrovascular response to simulated diving in the rat using the cerebral blood flow (CBF) tracer [14C]-isopropylidoxapentamine (IPIA) and quantitative autoradiography. IPIA was infused during simulated diving elicited by nasal water flow plus apnea in anesthetized rats. Autoradiographic brain images were scanned by a computer-based image analysis system (Image 1, Universal Imaging Corp.) and CBF was determined in absolute terms (ml min^-1100g^-1). Rates of blood flow were pseudo-color coded and displayed as a color image. During simulated dives of 30 seconds while brain blood flow was maintained or elevated without any regional reductions in CBF, Regional changes in blood flow (rCBF) are changes proportional to regional metabolic activity. A change in rCBF may indicate a change in neural activity. If this assumption is true, this technique could potentially be useful in determining the integration of different cardiovascular responses during diving in the central nervous system (CNS). Preliminary results suggest that the CBF is maintained and elevated during simulated diving despite the significant associated decrease in cardiac output.

Research supported by NSERC of Canada.

9.39
Avian renal adaptations to different environments. Giovanni Castorina and Ken L. Johnson, Division of Animal Sciences, University of Arizona, Tucson, AZ, 85724; School of Veterinary Studies, Murdoch University, Murdoch, Western Australia 6150.

This study examined the renal morphological adaptations both quantitatively and qualitatively, of Australian honeyeaters inhabiting either arid or wet zone environments. The volume of the kidney, as well as the volume of the upper part of the kidney and ureteric subcomponents was determined using stereology. The length of the loops of Henle and the percentage of looped and loopless nephrons were also determined. Renal ultrastructure was examined and compared using transmission electron microscopy. Arid zone species had a significantly higher volume of renal medulla than did wet zone species. There were no significant differences in either the volume or surface area of nephron subcompartments. AVT concomitantly had a higher proportion of looped nephrons and significantly longer loops of Henle than did wet zone species. Ultrastructurally, adjacent proximal tubules cells of arid zone species had wide intracellular spaces, filled with intesitnted infoldings. These were absent in wet zone species. All of the above characteristics indicate that arid zone honeyeaters have the potential to produce more hyperosmotic urine than wet zone honeyeaters. In cross section, all honeyeaters had the descending and ascending limbs of Henle separated by the collecting ducts. This spatial arrangement contradicts the current theory of urine concentration in birds.

Research supported by N.E.R.C. grant GR9/0065.

9.41
Na/K-ATPase is functionally coupled to creatine kinase in gill cells of an inflow fish. Darrell Kihara and George N. Somero, Ocean Sciences, Stanford University, Standford, CA 94305.

We demonstrate that the short-term mechanisms underlying the ability of the euryhaline fish Gillichthys mirabilis to tolerate large and rapid salinity fluctuations are not only passive but include a complex evolutionary adaptation of the gill energy metabolism. Despite a significant disturbance and very rapid regulation of the plasma osmolality after a transfer of this species from 36 ppt to 60 ppt within 6 hours, the ATP content and the number of Na/K-ATPase pumps did not change. In contrast, the creatine content increased significantly. The creatine content of gill cells was 10 to 40 times higher than the ATP content and the total specific activity of the creatine kinase (CK) exceeded that of the Na/K-ATPase 3 to 5 times. Using native metabolic coupling between CK and Na/K-ATPase is given in permeability subcomponents was determined using stereology. The length of the loops of Henle and the percentage of looped and loopless nephrons were also determined. Renal ultrastructure was examined and compared using transmission electron microscopy. Arid zone species had a significantly higher volume of renal medulla than did wet zone species. There were no significant differences in either the volume or surface area of nephron subcompartments. AVT concomitantly had a higher proportion of looped nephrons and significantly longer loops of Henle than did wet zone species. Ultrastructurally, adjacent proximal tubules cells of arid zone species had wide intracellular spaces, filled with intesitnted infoldings. These were absent in wet zone species. All of the above characteristics indicate that arid zone honeyeaters have the potential to produce more hyperosmotic urine than wet zone honeyeaters. In cross section, all honeyeaters had the descending and ascending limbs of Henle separated by the collecting ducts. This spatial arrangement contradicts the current theory of urine concentration in birds.

Research supported by N.E.R.C. grant GR9/0065.

9.40
GLOMERULAR FILTRATION OF FITC-DEX IN CHICKENS AND RATS. Stefani L.B. Boykin, Richard C. Schaffter, and Eldon J. Braden, Department of Physiology, University of Arizona, Tucson, AZ, 85724.

Avian ureteral urine contains a large amount (3-15 mg/ml) of protein that ranges in molecular weight from 20 - 120 kDa. Data from mammals suggests that the selectivity of the glomerular barrier would prevent filtration of all but the smallest of these proteins, thus, it was of interest to know the origin of the proteins in avian urine. The aim of the present study was to compare the size selectivity of the avian glomerular filtration barrier to that of its mammalian counterpart. This was accomplished by simultaneously infusing a heterogeneous mixture of fluorescein isothiocyanate–dextran (FITC–Dex) and a fluorescein–inulin (for GFR determination) into chickens (white Leghorn, 5-8 months old) and rats (Munich Wistar rats). The average molecular weights of the FITC–Dextran were 3, 10, 19, 40, 70, and 125 kDa (molecular radii, 15, 23, 30, 45, 58, and 83 Å). The FITC–Dextran present in urine and plasma were separated by size-selective HPLC and quantified with a fluorescence spectrophotometer–computer detection system. Size selectivity of the filtration barrier was determined from the fractional clearances of FITC–Dextran. Results from both species indicated that the glomerular filtration barrier approaches zero at an a of 64Å. Although there are no previous data for chickens, it has been reported for mammals (Munich Wistar rats) that the size selectivity of the glomerular barrier approaches zero at an a of 44Å. The data from our study suggest that some of the protein found in avian ureteral urine may be freely filtered at the glomerulus, but raises the further question of why similar proteins do not have the same filtration characteristics at the mammalian glomerulus. NSF (NIH 2290241) VA merit review grant to RCS.

9.42
MODULATION OF BRANCHIAL ION MOVEMENTS BY RAINBOW TROUT AT HIGH pH. Michael P. Wilkie, Pierre Largent and Chris M. Wood, McMaster University, Hamilton, CANADA. Centre National de La Recherche Scientifique, Strasbourg, FRANCE.

Exposure of salmonids to alkaline pH (pH > 9.0) leads to significant, sometimes lethal, reductions in plasma electrolytes (1,2). Accordingly, the primary goal of the present investigation was to use radioisotopes ("Na", "Cl") to relate changes in unidirectional ion movements across rainbow trout gills during a 72 h pH 9.2 exposure regime, to alterations in internal Na+ and Cl- balance and branchial ion transporter number and affinity. Blood volume was lowered by hemorrhage (10% of blood volume), 60 minutes after this procedure circulating AVT was unchanged. In subsequent groups blood volume was expanded by the infusion of 150 mN NaCl (30% of blood volume) for 30 minutes and resulted in significantly larger plasma AVT concentrations when compared with non volume expanded animals (5.3±0.6 vs 4.5±0.6 pg/ml, p<0.05). Plasma osmolality was experimentally increased by intraperitoneal (IP) injection of 1 M NaCl (0.5ml/100g R.W.) compared with 150 mM NaCl injected control fish (320.4±3.4 vs 320.4±3.0 mOsmol/kg,H2O). Plasma AVT concentrations 60 minutes after IP hypertonic saline injection were raised (6.4±1.1 vs 4.0±0.2 pg/ml, p<0.05). These results indicate that circulating AVT levels may be influenced by changes in blood volume and plasma osmolality in SW adapted fish.

Research supported by N.E.R.C. grant GR9/0065.
Moreover, 28Mg transport was cis- and trans-stimulated by Sti-Evans. University of Florida, Gainesville, FL. 32611
MYXINE GLOUTINOSA, yes TOOT>*. John A Donald*, AND David H. Klaus W. Beyenbach. Cornell University, Ithaca, NY 14853
AGLOMERULAR EURYHALINE TELEOST. Mark D. Baustian and OSMOCONFORMATION AND IONOREGULATION IN AN
Submitted in part by the Max-Planck-Gesellschaft, Alexander-
in the kidneys of freshwater adapted fish.
the inhibitors verapamil and diltiarem had no effects.
cations (20 mM) according to a selectivity sequence predicted
Membrane Toxicology at M.D.I. Biological Laboratory, Maine.
to the voltage generated by Na/D-glucose cotransport
that the Mg pathway studied here is located in apical BBM of
phosphatase (5.5-fold) and y-glutamyl transpeptidase (1-fold).
in plasma, the osmotic pressure of bladder urine decreases from 295±23 mOsm to a new steady state of 242±23 mOsm
within 7 days. Plasma Na and Cl concentration also decreased but
only 50% and urine [Na] increases 3.7-fold. The volume of spontaneously
environmental Mg loading, urine [Cl] decreases by 81% indicating
of Mg, in SW, to predominately sulfur salts of Na, in 10% SW. Following
composition of bladder urine changes from predominately SO4 and Cl salts
competed for 50 % of 125I-ANP sites. C-ANF failed to compete
over 500 million years ago. Natriuretic peptides (NPs) are
osmoconforming agnathan that diverged from other vertebrates
Gly22[ANP-(4-23)]-NH2 (C-ANF, which binds to the mammalian and
receptors (NPRs) in the myxinoid kidney were examined to
over 500 million years ago. Natriuretic peptides (NPs) are
osmoconforming agnathan that diverged from other vertebrates
the renal loss of solute the toadfish lowers plasma osmotic pressure.
used in tissue section autoradiography, competition studies, and
guanyle cyclase (GC) assays. Rat atrial and porcine C-type
NATRIURETIC PEPTIDE RECEPTORS IN THE KIDNEY OF THE AGNATHAN,
the higher vertebrate CNP and 'clearance' types are absent in
absence of a short term RVD evident from both sets of measurements, tissue
taurine, betaine, and K+ content remained unchanged during these
intracellular water in animals acclimated for 2 weeks to 60% ASW was still
higher (0.43 ml/g) than in mussels maintained in 100% ASW, and tissue
increased from 0.36 ml/g wet wt in 100% ASW to 0.44 ml/g after 3 min in
200×30 mU/mW on 7 days. Plasma Na and Cl concentration also decreased but
hemocrit did not change, suggesting that the decrease in osmotic pressure is
due to salt loss rather than water loading. In parallel with the changes in plasma, the osmotic pressure of bladder urine decreases from 295±23 to
205±30 mOsm with no significant change in the U/P ratio. The
composition of bladder urothelium is predominately SO4 as Mg, in SW, to predominantly sulfur salts of Na, in 10% SW. Following transfer, urine [Mg] decreases by 85% reflecting a decrease in environmental Mg loading, urine [Cl] decreases by 81% indicating
continued renal reabsorption, while total urine sulfur levels decrease by only 50% and urine [Mg] increases 3.7-fold. The volume of spontaneously
voided urine increases from 17.6±4.3 to 31.6±2.3 μl/100g. In response to
hyposmotic stress, toadfish increase urinary water loss but are unable to
prevent concomitant solute loss. We conclude that the inability of the
toadfish renal system to produce a urine hyposmotic to plasma forces the
toadfish to lose solute in response to hyposmotic challenge. To minimize
the renal loss of solute the toadfish lowers plasma osmotic pressure.
9.45
OSMOCONFORMATION AND IONOREGULATION IN AN
AGLOMERULAR EURYHALINE TELEOST. Mark D. Baustian and
University of Maine, Orono, ME 04469-5735
We have examined the composition of plasma and urine from the
toadfish (Opsanus tau) for up to 60 days following abrupt transfer from full strength seawater (SW) to 10% SW. Plasma osmotic pressure rapidly
decreases from 320±16 mOsm to a new steady state of 242±30 mOsm
within 7 days. Plasma Na and Cl concentration also decreased but
hemocrit did not change, suggesting that the decrease in osmotic pressure is
due to salt loss rather than water loading. In parallel with the changes in plasma, the osmotic pressure of bladder urine decreases from 295±23 to
205±30 mOsm with no significant change in the U/P ratio. The
9.46
ABSENCE OF VOLUME REGULATION IN RESPONSE TO HYPO-
OSMOTIC SHOCK IN GILL CELLS OF I Myxinus. P.S. Neufeld A . W. Wr@ Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724
Unlike most animal tissues, cells in the gill of the mussel, Mytilus, californianus, did not typically respond to an acute hyposmotic shock with a
volume regulatory decrease (RVD). Two techniques showed that gill cells from mussels acclimated to 100% artificial seawater (ASW) remained
swollen for at least 1 hr following exposure to 60% ASW. First, the height
of ciliated lateral cells was measured microscopically. Cell height increased by
20% when challenged with 60% ASW and generally remained at 20% above control for 1 hr, although a few cells showed a modest RVD during this
period. Second, total intracellular water was calculated for intact gills
bathed in, and perfused with, ASW containing 31O2 (for total water space) and
14C-polyethylene glycol (for extracellular space). Intracellular water
increased from 0.36 ml/g wet wt in 100% ASW to 0.44 ml/g after 3 min in
60% ASW, and was 0.47 ml/g after 1 hr in 60% ASW. Consistent with the
absence of a short term RVD evident from both sets of measurements, tissue
urine, betaine, and K+ content remained unchanged during these
treatments (-500, 200, and 220 μmol/g dry wt, respectively). In
fact, intracellular water in animals acclimated for 2 weeks to 60% ASW was still
higher (0.43 ml/g) than in mussels maintained in 100% ASW, and tissue
urine, betaine, and K+ content remained unchanged. Absence of an RVD
in tissues from intestial mussels would allow these animals to avoid the
energetically expensive solute fluxes required for volume regulation during
exposure to cyclic fluctuations of salinity. (NSF award DCB88-19367 and
NIH training grant HL-07249)
9.47
NATRIURETIC PEPTIDE RECEPTORS IN THE KIDNEY OF THE ANTHAN,
WILLIAM GLUTONUS. Tag-Toom J. Donald* AND David E. Under, University of Florida, Gainesville, FL 32611
The Atlantic hagfish, Myoxine glutonius, is a marine neomoform that diverged from other vertebrates over 600 million years ago. Natriruepic peptide (NPs) are
inolved in salt and water homeostasis in mammals and are homologous in neomorphs. Natriuretic peptide receptors (NPRs) in the myxinoid kidney were examined to
establish their relationship with higher vertebrate NPRs. In the Atlantic hagfish, NPR-C (125I-NPR-C) was used in tissue section autoradiography, competition studies, and
guanyle cyclase (GC) assays. Rat atrial and porcine C type
expression. This study suggests that GC-linkage of NPRs is an ancient verrtebrate characteristic, and that NPRs similar to the
higher vertebrate NPR and 'clearance' types are absent in the hagfish kidney; furthermore, the observed NPR appears similar to the mammalian NPR C-linked. Supported by NPR
DCB 951643 to DW, and NIH HD-3370-ES08226 to the Center for Membrane Toxicology at M.D.I. Biological Laboratory, Maine.
9.48
EXPRESSION OF THE MYOLOBIN GENE IN ANTARCTIC CHANNICHYID
ICEFISH. Deena Small-Barrv, Bruce D. Sidell and Michael E. Vavda.
University of Maine, Orono, ME 04469-5735
The Antarctic icefish are the only known vertebrates that lack
hemoglobin as adults, and there is controversy in the literature whether they lack myoglobin (Mb) expression as well. Using
radioactive probes, we demonstrated that gill cells from mussels acclimated to 100% artificial seawater (ASW) remained
swollen for at least 1 hr following exposure to 60% ASW. First, the height
of ciliated lateral cells was measured microscopically. Cell height increased by
20% when challenged with 60% ASW and generally remained at 20% above control for 1 hr, although a few cells showed a modest RVD during this
period. Second, total intracellular water was calculated for intact gills
bathed in, and perfused with, ASW containing 31O2 (for total water space) and
14C-polyethylene glycol (for extracellular space). Intracellular water
increased from 0.36 ml/g wet wt in 100% ASW to 0.44 ml/g after 3 min in
60% ASW, and was 0.47 ml/g after 1 hr in 60% ASW. Consistent with the
absence of a short term RVD evident from both sets of measurements, tissue
urine, betaine, and K+ content remained unchanged during these
treatments (-500, 200, and 220 μmol/g dry wt, respectively). In
fact, intracellular water in animals acclimated for 2 weeks to 60% ASW was still
higher (0.43 ml/g) than in mussels maintained in 100% ASW, and tissue
urine, betaine, and K+ content remained unchanged. Absence of an RVD
in tissues from intestial mussels would allow these animals to avoid the
energetically expensive solute fluxes required for volume regulation during
exposure to cyclic fluctuations of salinity. (NSF award DCB88-19367 and
NIH training grant HL-07249)
INTRACELLULAR FATTY ACID-BINDING PROTEIN FROM THE ANTARCTIC ICEFISH Chaenocephalus aceratus. MAINTENANCE OF BINDING CHARACTERISTICS AT LOW BODY TEMPERATURE. Richard L. Londero* and Bruce D. Siddel. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950 and University of Maine, Orono, ME 04469.

Fatty acid-binding proteins (FABPs) are a family of cytosolic proteins that bind hydrophobic ligands (primarily long-chain fatty acids) by a combination of hydrophobic and ionic interactions. Both types of bonds are temperature sensitive, therefore the role of FABPs in membrane fluidity is still under investigation. We characterized a FABP from a cold-living animal, and compared its physical and physiological properties to those of a mammalian FABP. We purified the FABP from the aerobic muscular pectoral muscle of C. aceratus (CA-FABP) and found that it is remarkably similar to homologous mammalian FABPs.


Previously we measured different concentrations of LDH-B in the resting cardiac glycolytic flux of two cod populations. The resting cardiac glycolytic flux of these populations in order to investigate whether these differences in enzyme concentration correlate with differences in higher-order physiological processes. We acclimated fish from two populations, Maine and Georgia, to temperatures near their thermal maxima by delivering directly to hearts in flasks containing NaHCO3/temperature. Glycolytic flux was determined by elution of the enzyme from the cardiac muscle and in a liquid scintillation counter to determine 3H2O production. Since the postcardiomyocyte is negligible in these hearts, glycolytic flux can be calculated from the amount of tritiated water produced. We found no differences in resting flux rates between populations at acclimation temperatures (pH=7.4 in both cases). This indicates that variation in enzyme concentrations is not reflected in the resting cardiac glycolytic rate. However, we cannot rule out the possibility that the protein concentration in enzyme concentration may correlate with glycolytic flux under other conditions, such as anaerobic or aerobic exercise.

9.52 SEASONAL CHANGES IN LEVELS OF UBQUITIN CONJUGATES AND HSP 70 IN INDIVIDUAL MUSCLE CELLS. E.J. Heumann and George N. Somero. Oregon State University, Corvallis, OR 97331-2914.

We examined the effect of environmental temperature on protein damage in a natural population of the intertidal mussel, Mytilus californianus. In order to compare the state of protein pools during seasonal variations in environmental temperature, we measured ubiquitin (Ub) conjugate levels and relative quantities of the stress protein hsp70 as indicators of environmental-induced protein damage. Ubiquitinated proteins are irreversibly damaged and are degraded by intracellular proteasomes, stress proteins are heat-inducible and known to re-fold denatured proteins. Gill tissue samples were collected and measured using two body temperature waters. During the field season, the cold-water period, the northern fish express twice as much LDH-B protein as the summer-acclimated mussels. The results showed that significant differences in the two biochemical indicators correlated with a 20°C difference in muscle temperature. In cold collected from temperature-acclimated mussels, the Ub conjugate levels were 5-6 times higher than those measured in winter-acclimated mussels. Similarly, the relative expression of hsp70 was highest in summer-acclimated mussels. The seasonal differences in the two biochemical indicators of protein damage suggest that environmental temperature has a strong impact on the state of protein pools in intertidal organisms. Given the energetic cost to replace damaged proteins, environmentally-induced protein damage may have an impact on whole animal energetics.

9.53 KINETICS OF GORY LACTATE DEHYROGENASES IN RELATION TO ENVIRONMENTAL TEMPERATURE. Peter A. Fields, George N. Somero and Jeffrey B. Graham. Scripps Inst. Oceanography, UCSD, La Jolla, CA 92037.

Lactate dehydrogenase (LDH) kinetics of four species of gobies in the genera Gillichthys and Cotyphopterus were studied across a broad range of temperatures to determine how the enzyme has adapted to different environmental conditions. Because LDH is important in anaerobic metabolism, it should be under strong adaptive pressures to maintain appropriate kinetic properties when confronted by different thermal regimes. In this study, we compared LDH from a cold-stenothermal goby (C. nicholsi) to those from a warm-stenothermal goby (C. personatus) and two species of eurythermal goby (G. seta, G. mirabilis). We purified LDH from each species were determined at 5 degree intervals from 10 - 40°C. The kinetic properties are not significantly different from values measured for rat heart-LDH at 25°C in part assays. However, CA-FABP does have significantly less hydrophobic binding pocket than rat heart FABP, as measured by relative quantum yield of a fluorescent fatty acid analog. Hydrophobic interactions, which are destabilized at low temperature, thus appear to contribute less to total bond strength and may be less important in FABPs from cold-adapted species than in FABPs from better-adapted species.


Northern populations of the teleost fish Funduloides heteroclitus are subjected to colder waters than southern populations. In a compensatory fashion, the northern fish express twice as much LDH-B protein as southern fish due to an increase in their Ldh-B transcription rate. Initial characterization of the 5' regulatory region indicated that there was a significant amount of sequence variation between these populations. Some of this variation appeared to be functionally important in that it was correlated with regions that influence LDH-B transcription. Both of these bottleneck motifs are appropriate choices for indicators of protein damage. Ubiquitinated proteins are irreversibly damaged and are degraded by intracellular proteasomes, stress proteins are heat-inducible and known to re-fold denatured proteins. Gill tissue samples were collected and measured using two body temperature waters. During the field season, the cold-water period, the northern fish express twice as much LDH-B protein as the summer-acclimated mussels. The results showed that significant differences in the two biochemical indicators correlated with a 20°C difference in muscle temperature. In cold collected from temperature-acclimated mussels, the Ub conjugate levels were 5-6 times higher than those measured in winter-acclimated mussels. Similarly, the relative expression of hsp70 was highest in summer-acclimated mussels. The seasonal differences in the two biochemical indicators of protein damage suggest that environmental temperature has a strong impact on the state of protein pools in intertidal organisms. Given the energetic cost to replace damaged proteins, environmentally-induced protein damage may have an impact on whole animal energetics.

Supported by a NSF Marine Biodiversity Research Postdoctoral Fellowship to GEH and NSF grant IBN 92-66060 to GNS, and NSF grant IBN 92-65734 to JRH and IBN-93-07042 to CAR.

The physiological properties of adenosine may be essential in the control of energy metabolism for the survival of animals exposed to oxygen shortages. Accordingly, we tested the hypothesis that adenosine mediates the response of rainbow trout to hypoxia. Treatment of hypoxic rainbow trout (P<0,25 torr) with the adenosine receptor (AR) blocker theophylline (4 mg/Kg) increased the concentration of blood and tissue lactate above their hypoxic controls. This response was associated with the rapid development of a metabolic acidosis. Compared to normoxic animals, decreases in creatine charge were only observed in the heart and red muscle, but not white muscle, of theophylline treated fish. The glycogen content of the heart also decreased following AR blockade. The tissue metabolites of trout treated with enprofylline, an AR blocker with very weak affinity, were similar to the hypoxia sham fish. Both AR blockers had no measurable effects on normoxic controls. Following theophylline treatment, increased circulating concentrations of adrenalin and cortisol, and reduced splenic contribution of rbc to the circulation, may both contribute to the hypoxic response observed with AR blockade. Supported by NSERC and the Science Council of B.C.


Confinement and crowding stresses cause gulf toadfish to switch from ammoniogenically to nearly complete ureoxygen over short periods (e.g. 24 h). We examined the effects of gulf stressors, including up-regulating hepatic glauconate synthase (GNS), a key rate-limiting enzyme in toadfish ureogenesis, by blocking cortisol synthesis with metyrapone. Toadfish injected with saline and stressed (crowded) for 24 h had elevated cortisol and GNS (+482% and +209% respectively), while the response in similarly stressed metyrapone injected fish was significantly depressed (e.g. cortisol: +179%: GNS: +1%). Metyrapone or saline injected fish returned to large tanks with cover had modest elevations of cortisol (192:220%) and GNS (126:145%) which were not significantly different from one another. Chronically stressed toadfish (+72 h) exhibited high cortisol levels (+632%) but only moderately elevated GNS activities (+151%). While initially ureogenic, these fish showed an extreme stress response wherein total nitrogen excretion surged with ammonia as the greatest fraction. Our results show that cortisol is important in initiating ureogenesis but at very high levels it overrides the urinogenic response presumably by mobilizing fuels (e.g. glucose, amino acids) and thyroid hormones which alter intermediate metabolism. Supported by NSERC and NSF (IBN 9111819).

9.57 SUPPRESSION OF THE UBIQUITIN-MEDIATED PROTEOLYTIC PATHWAY IN QUIESCENT ARTEMIA EMBRYOS.

Thomas J. Anchordoguy* and Steven G. Hand. Univ. Colorado, Boulder, CO 80309

Many organisms withstand adverse environmental conditions by entering a reversible state of quiescence that may last for months or years. In this study we present evidence that the reduction in oxygen energy status and the associated intracellular activities occurring during quiescence-induced quiescence could be directly or indirectly, the initiating step in the ubiquitin-mediated proteolytic pathway in embryos of the brine shrimp Artemia franciscana. The levels of ubiquitin-conjugated proteins drop to 59% of control (serum) values during the first hour of anoxia, and near 7% in 24 h. ATP falls to 5% of normal values under anoxia and AMP rises reciprocally. This energy limitation is accompanied by a significant depression of intracellular pH (pH). By comparison, when embryos are subjected to artificial acidosis under aerobic conditions (pH, drops sharply, but ATP does not change for hours), ubiquitin-conjugated proteins decline to 55% after 1 h. Additional studies on recovery from 24 h anoxia or acidosis indicate that the levels of ubiquitin conjugates and ATP rapidly increase and AMP levels decrease upon the return of embryos to aerobic conditions. Furthermore, when anoxic embryos are exposed directly to aerobic acidosis, 71% of the total suppression of ubiquilatin is maintained despite the return of embryos to control levels. Thus, while elevated AMP and severely depleted ATP may contribute to the arrest of ubiquitin conjugation, intracellular acidification appears to play the predominant role. We conclude that the arrest of ubiquilatin synthesis likely serves to suppress ubiquitin-mediated degradation of protein, thereby preserving macromolecular integrity and potentially explaining the remarkable extension of protein half-life observed under anoxia in these embryos. [Supported by NSF grant IBN 9005653].


The purpose of this study was to compare levels of plasma epinephrine (EPI) and norepinephrine (NE) in bottlenose dolphin acclimated to 25°C and 15-20°C with those in dolphin, COOL WATER. M. Heath, S. Ridmay, M. Malik, J. Thomas and W-G. Milier. Naval Medical Research Institute, Bethesda, MD 20889-4607.

The glycogen content of the heart also decreased following AR blockade. The levels of plasma NE in dolphin acclimated to 25°C and 15-20°C were 97.1±65.8 (mean±std; 3 samples) and 10.1, respectively. The pre-transport (baseline) levels of dopamine (DA), norepinephrine (NE) were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. Thus, both catecholamines were measurable increases in plasma NE in dolphin occur even when the responses to a low temperatures that disabpears with acclimation to the-new thermal environments. The mean values during the first hour of anoxia, and near 7% in 24 h. ATP falls to 5% of normal values under anoxia and AMP rises reciprocally. This energy limitation is accompanied by a significant depression of intracellular pH (pH). By comparison, when embryos are subjected to artificial acidosis under aerobic conditions (pH, drops sharply, but ATP does not change for hours), ubiquitin-conjugated proteins decline to 55% after 1 h. Additional studies on recovery from 24 h anoxia or acidosis indicate that the levels of ubiquitin conjugates and ATP rapidly increase and AMP levels decrease upon the return of embryos to aerobic conditions. Furthermore, when anoxic embryos are exposed directly to aerobic acidosis, 71% of the total suppression of ubiquilatin is maintained despite the return of embryos to control levels. Thus, while elevated AMP and severely depleted ATP may contribute to the arrest of ubiquitin conjugation, intracellular acidification appears to play the predominant role. We conclude that the arrest of ubiquilatin synthesis likely serves to suppress ubiquitin-mediated degradation of protein, thereby preserving macromolecular integrity and potentially explaining the remarkable extension of protein half-life observed under anoxia in these embryos. [Supported by NSF grant IBN 9005653].

10.2 BIOSYNTHETIC APPLICATIONS OF MARINE MAMMAL PHYSIOLOGY


The purpose of this study was to compare levels of plasma epinephrine (EPI) and norepinephrine (NE) in bottlenose dolphin acclimated to 25°C with those in dolphin acclimated to 15-20°C and to measure changes following a rapid transition from 25°C to 20°C water. Nine dolphin, 3 in Hawaii and 6 in San Diego, were used. All dolphin had been accustomed to blood sampling procedures. Blood samples were collected from the caudal peduncular vein into heparinized tubes, immediately centrifuged at 600 g for 10 minutes and plasma stored at 0°C until assayed. The concentrations of EPI and NE were measured by RIA with electrochemical detection. The levels of plasma EPI in dolphin acclimated to 25°C and 15-20°C were 97.1±65.8 (mean±std; 3 samples) and 127.5±32.2 pg/ml (12 samples, respectively). The levels of plasma NE were 636.0±122 pg/ml and 10.1, respectively. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml. On the day after transport, plasma NE rose to 10.1, but not significantly lower in this small sample. The pre-transport (baseline) levels of NE were 636.0±122 pg/ml.
11.3

EXTRACELLULAR Ca²⁺ EFFECT ON BASOLATERAL TETRA-
ETHYLMORTONIUM (TEA) TRANSPORT IN ISOLATED SNAIL RENAL
PROXIMAL TUBULUS. Y. K. Kim and W. D. Andrus, Dep. of Physiol.,
Cot. of Med., Univ. of Ariz., Tucson, AZ 85724.

Ca²⁺ is an important determinant of a variety of cell functions. We have examined the effect of the removal of extracellular Ca²⁺ on basolateral TEA uptake and efflux in isolated snail renal proximal tubules (25°C; pH 7.4). 0M Ca²⁺ (Ca⁰), produced by adding 0.2M EGTA to 0M Ca²⁺ Ringer-solution, increased the 2mm of uptake of [H]TEA by about 18%. In addition, the efflux coefficient for [H]TEA was increased by 44% (17.3±1.7 vs. 8.9±0.7 ml/min/mg for control, p<0.05). The cell interior was acidified by 0.22±0.05 pH U and the basolateral membrane potential was depolarized by 27mV with 0M Ca²⁺ (Ca⁰). However, our previous data show that depolarization reduced TEA uptake and had no effect on I/E efflux. Intracellular acidification with 0M Ca²⁺ might have accelerated TEA uptake via enhanced H⁰/TEA countertransport. However, this process would not enhance TEA efflux with 0M Ca²⁺ (Ca⁰). Moreover, during 20M NH₄CI pulse experiments, intracellular acidification (6.9±0.04 vs. 7.29±0.06 pH U for control) did not increase TEA uptake (24.1±2.53 vs. 21.59±2.27 fmol/min/mg, p=0.05) whereas intracellular alkalization (7.7±0.07 vs. 7.94±0.06 pH U) did increase TEA uptake by 24% (18.99±3.42 vs. 15.34±2.63 fmol/min/mg, p<0.05). During acidification with NH₄CI pulse, 0M Ca²⁺ (Ca⁰) did not have an effect on TEA uptake (35.10±7.94 vs. 32.31±5.39 fmol/min/mg for control, p>0.05). Thus, 0M Ca²⁺ (Ca⁰) had a greater effect on basolateral TEA efflux than on uptake. These results cannot be explained by changes in intracellular pH or membrane potential. (NSF DCR 900185).

11.4

IONIC COMPOSITION OF CHICK EMBRYO AIRWAY LIQUID RESEMBLES
AMNIOTIC FLUID. Bob McCulough, Amy Montgomery, and Thomas A. Davis,
Department of Biology, Loyola College, Baltimore, MD 21210.

Sheep fetal lung liquid has higher chloride ion concentration and total osmolality compared to amniotic fluid. Previous studies have shown that most mammalian fetal lung uses active Cl⁻ ion transport to secrete their own fluid the volume of which is critical to postnatal lung function. Avian embryonic lungs and air sacs are also fluid-filled but the site of origin, the ionic composition and the physiological role of this fluid have not been described in the past. Chicken eggs were opened on days 14, 16, and 18 of incubation and samples of amniotic fluid and airway fluid were removed by syringe. Fluid samples were analyzed for total osmolality and chloride concentration.

<table>
<thead>
<tr>
<th>DAY</th>
<th>Tot Osm</th>
<th>[Cl⁻]</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>116.0 ± 7.8</td>
<td>258.4 ± 9.8</td>
</tr>
<tr>
<td>16</td>
<td>115.0 ± 10.2</td>
<td>241.0 ± 9.3</td>
</tr>
<tr>
<td>18</td>
<td>115.0 ± 10.2</td>
<td>241.0 ± 9.3</td>
</tr>
<tr>
<td>Sheep</td>
<td>157.0 ± 4.1</td>
<td>234.0 ± 2.0</td>
</tr>
</tbody>
</table>

These results show that the [Cl⁻] and total osmolality of amniotic fluid and airway fluid are not significantly different. The chloride content of amniotic fluid is increased during incubation and may be important in its production and development of the avian air sacs and lungs. This research was supported by a grant to JAU from the Iowa Academy of Science.
A geometric model of the pituitary secretion of the hypothalamo-pituitary axis. We develop a comprehensive dynamical model of the neurosecretory system of hypothalamo-pituitary-adrenal axis. The mathematical formulation of it is

\[
\frac{dE}{dt} = \frac{1}{1 + 10^{(E - 1500)}} / \frac{dP}{dt} = \frac{1}{1 + 10^{(P - 1500)}}
\]

where \(E\) denotes respectively the plasma concentration of corticotropin-releasing hormone (CRH), corticotropin (ACTH), free cortisol, CBG-bound cortisol, and albumin-bound cortisol. The unit of all \(x, y\) are \(mg\), time unit is minute. Some results derived from this model and their corresponding experimental results are given in the following table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CRH</th>
<th>ACTH</th>
<th>Free cortisol</th>
<th>CBG-bound cortisol</th>
<th>Albumin-bound cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Abnormal</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

From this table, we see the deductions of our model are in good agreement with the experimental results.

12.5


Cnr, Omaha, NE 68198.

Large members of a species usually have larger brains than the small members; the larger sex in dimorphic species usually has the larger brains; with enhanced nutrition, the animals grow larger and have larger brains. The sample of Pocket gophers trapped by Patton and Brylski (Amer. Nat., 1987, 130:493-506) in desert shrubland and in an irrigated alfalfa field show all three conditions. They measured the body weight (P), body length (L) and cranial basilar length (Z) of each specimen, and we measured cranial capacity (E) determines its allometric variation with P, L, and Z. In both sexes from both sites, their within-species E/P and E-Z/2 slopes were similar, but their E-Z/2 slopes were steeper. Their between-sex slopes were nearly equal, and similar to their between-group slopes of 1/18th-1/6th. We argue that the between-group slopes show the AE needed for the brain to stay in "propor" proportion when a body changes by DT. A geometric model that uses the statistical characteristics of the sample was used to predict the between-group slopes from the desert shrubland sample. It predicted accurately.
13.1 EFFECTS OF ENVIRONMENTAL pH AND CALCIUM ON THE PHYSIOLOGY AND BEHAVIOR OF FRESHWATER SNAILS, Mary Lou Fiadil, Jack W. Fentemietla, and Raymond P. Henry, Auburn University, Auburn, AL 36849

Freshwater snails, often used as indicators of adverse environmental conditions, are extremely sensitive to acidification. Several correlative studies have documented decreased growth in small acidified streams receiving acidic inputs. However, we know little about the specific physiological effects of acidification, and how essential ions, such as calcium, affect physiological resilience of snails. For example, growth rates (Gompertz parameters), survival rates, and calcium levels, in streams of the southeastern United States, was exposed to a wide range of pH and calcium levels to examine blood chemical responses to acidification. We exposed snails in the laboratory for 72 hr to pH levels 4, 5, 6, and 7 (control), and we supplemented each pH level with 0, 5, 0.5, or 50 mg/L CaCl2. Hemolymph was collected at 0, 3, 6, 12, 24, 48, and 72 hr, and analyzed for pH, HCO3, Ca, Na, and K. All blood factors differed among environmental pH levels (P < .0001) and these differences increased over 72 hr (P < .005). Blood HCO3 and blood pH were the only factors that were affected by environmental Ca (P <.05), which suggested that uptake of environmental Ca is (1) essential for the regulation of blood ions at less toxic pH levels. Snails at pH 4 were completely inactive over 72 hr, but at pH 5, small activity strongly varied with environmental Ca level (i.e., 40% activity at 0 mg/L Ca, 69% at 5 mg/L Ca, and 90% at 50 mg/L Ca). These results suggest that environmental Ca is (1) essential for the regulation of blood ions at less toxic pH levels (P > 4), and (2) responsible for maintenance of normal activity levels.


Endothelin (ET) is now considered to be the most potent vasoconstrictor known, but the role of ET in fishes is relatively understudied. ET-1 previously has been shown to contract vascular smooth muscle (VSM) rings from both trout and catfish mesenteric arterics and cardinal veins. The trout ventral aorta contracted in one study, but was refractory in another. We investigated the effect of ET-1 on VSM from the trout ventral aorta to ET and characterized the receptor involved by means of relative sensitivity to ET-1 vs. ET-3 and the antagonist BQ 123 (cyclo-D-[D-His5]-ET-1) on the ET-1 receptor. We produced a concentration-dependent contraction (EC50 = 15 nM) in the aortic VSM with or without the endothelium, suggesting that endothelin receptors are not present on the endothelium itself, contrary to what is found in mammals. ET-3 was nearly as consitry, suggesting that ETA rather than ETB receptors were involved. The fact that BQ123 did not inhibit the ET-1 induced contraction of the aorta supports this hypothesis. Thus, our preliminary data strongly suggest that functional ET receptors are indeed present in shark aortic VSM, display high sensitivity to the mammalian peptides, and probably arc of the ETB, rather than ETA type, contrary to the situation in mammals. (Supported by NSF IBN-919122 and NIH-NR00997 to DHE as well as UHS-30-ES03828 to the Center for MembraneToxicity Studies at MDIBL)


In vivo and in situ experiments were performed on the Atlantic hagfish (Typhion gnoma) (1) to characterize the levels of circulating catecholamines during acute stress, presumably tyramine, norepinephrine or epinephrine (as-expressed), and (2) to evaluate the potential mechanisms of catecholamine release from the major sites of storage, the systemic heart and posterior cardinal vein (PCV). Adrenaline and noradrenaline were stored at roughly equivalent concentrations in cardiac tissue, whereas noradrenaline was the predominant catecholamine stored in the PCV. Exposure of hagfish to acute hypoxia for 30 min caused significant increases in plasma noradrenaline levels, whereas adrenaline levels were unaffected. Exposure of fish to anoxia or physical disturbance (5 min of anoxia exposure) also elicited pronounced increases in plasma noradrenaline levels (6-10 times) and, to a lesser extent, adrenaline levels (2-3 times). An in situ saline perfused heart preparation was used in an attempt to elucidate the mechanism(s) underlying the stress-induced release of catecholamines from the chromaffin tissue of the heart and PCV. The ultrasound receptor agonist urapidil (10(-6)-10(-2) M) caused a significant increase of catecholamines at the site of similar mechanical stimuli on the human heart in vivo is doubtful because the hatchet heart is not thought to be innervated. Perfusion with anaerobic or acidic saline both failed to elicit catecholamine release. Further, the elevation of perfusion pressure to simulate a rise in venous pressure, as might occur during hypoxia or physical disturbance, was also without effect on release. The addition of pithetal to the following saline caused a marked release of catecholamines from the chromaffin tissue. Thus, the mechanism(s) of release of catecholamines from the heart of hagfish during stress in vivo remains unclear, although preliminary experiments suggest the possible involvement of pituitary hormones.


The Malpighian tubules of the house cricket, Acheata, are composed of two uniform regions, each with a very different cell type. The mid-tubule comprises about 75% of the total tubule length and consists of a single cell type. Fluid secretion was initiated 150-200 pl mm(-2) min(-1) and increased 3 fold following treatment with either cAMP or melatonin extracts of the corpora cardiaca (CC). Stimulated mid-tubules produced a sodium-rich, as opposed to the normally potassium-rich, fluid. These changes in secretion rate were accompanied by marked changes in ultrastructure, particularly in the morphology of the basolateral infoldings and the fine structure of the basal lamina. Notable changes were also seen in the morphology and position of the mitochondria, and a pronounced mobilization of the spherites, which are calcium phosphate granules embedded in the cells. Intracellular calcium concentrations were followed in single cells using fluorescence imaging. Treatment of whole tubules with both CC and cAMP produced increases in the concentrations of intracellular calcium, independent of the mobilization of the spherites. Supported by LEQSF grant RD-A-41 to JLS and USDA grant 9301630 to JHS and BEF.
13.7

EFFECTS OF THYROXINE ON METABOLISM AND ENERGY STORAGE IN THE MARINE TOAD, BUFO MARINUS. Kay Etheridge. Gettysburg College, Gettysburg, PA 17325

Chronic thyroxine (T4) supplementation in marine toads was accompanied by pellets implanted for three weeks; control animals received placebo pellets. A standard metabolic rate (SMR) was increased significantly (23%) in the T4-treated toads compared to controls. Activity of hepatic malic enzyme and hepatic-pyruvate kinase was unaffected by treatment, as was muscle and hepatic lactate dehydrogenase. Activity of hepatic malic enzyme and hepatic-pyruvate kinase was increased in T4-treated toads (75% and 70%, respectively); in muscle samples these enzymes were unaffected by treatment. T4 treatment increased hepatic malate dehydrogenase activity by 22% and hepatic glycogen phosphorylase activity by 1%. T4 increased liver triglyceride content 74%, but decreased hepatic triglyceride by 64%. Plasma glucose and triglyceride content were unaffected by treatment. T4 did elevate total plasma cholesterol significantly (74%). Among the enzymes examined, only hepatic enzymes appeared to be affected by treatment. These enzymes that showed an increase in activity are key regulators of aerobic glycolysis, lipid synthesis, and glycogen metabolism. Increased lipid synthesis could contribute to the increase in metabolic rate of these toads.

13.9


The reproductive cycle of a captive breeding colony of 30 female and 20 male desert tortoises was studied over a two year period at the Desert Tortoise Conservation Center, Las Vegas, Nevada. Blood samples were collected at monthly intervals via jugular puncture from each individual from April to October. The animals remained underground during the winter months. Plasma testosterone and corticosterone were measured in males and females, and plasma estradiol-17β and progesterone were measured in females using radioimmunoassay. Plasma estradiol was also measured as an index of vitellogenesis in females. Follicular growth occurred in the tail and was correlated with increased estradiol. Matings were observed in the spring and in the fall. Mating was observed from May to early July. Corticosterone was higher in males than in females in each month of the year. Plasma testosterone in males was highest in Aug.-Sept. (peak levels >200 ng/ml). Plasma testosterone in females was highest in April (ca. 6 ng/ml) and lowest in June and July (<1 ng/ml). Progesterone was low throughout the year except around the period of ovulation. Plasma estradiol showed two peaks, one in April and the second in Aug.-Sept. Estradiol and testosterone in the females did not show correlation.

24.1


Lizards are unable to run and breathe at the same time because their intercostal muscles assume a locomotor function during walking and running. To determine the phylogenetic extent of this locomotor role, I measured ventilatory air-flow with a mask-mounted screen photoge and activity of the fifth, sixth, and ninth intercostal muscles in four dogs walking and trotting on a modified treadmill. During rest and thermoregulatory panting, activity of the intercostal muscles was associated with inspiratory and expiratory airflow. However, during walking and trotting, activity of these muscles was correlated with locomotion. Activity of the external intercostals was associated with limb support by the ipsilateral forelimb, and activity of the internal intercostals was correlated with the suspension phase following ipsilateral front-support. When ventilation and stride were not synchronized, activity of the intercostal muscles stayed locked to the locomotor events but drifted in time relative to ventilation. Thus, the available evidence suggests that the primary function of the intercostal muscles ceases at the initiation of locomotion in both lizards and mammals. These observations, combined with data from the myotomal muscles of walking salmonids, indicate that locomotion was the ancestral function of the intercostal muscles. Support: NSF IBN 9258243 and IBN 9306466.

24.2


Magpie flight is characterized by high amplitude, short duration wingbeat cycles and low amplitude longer duration wingbeat cycles with intermittent glides. This provides an opportunity to study how variable flagging patterns may or may not affect respiratory pattern and mechanics. The flight-induced respiratory and wingbeat cycles of the magpie (Pica pica) were analyzed by video photogrammetry. Flight was categorized into two types: (1) flapping with glides and (2) flapping without glides. The flapping without glides was divided into two subcategories: (1) low amplitude longer duration cycles with intermittent glides and (2) high amplitude shorter duration cycles with no glides. The two types of flapping were analyzed in terms of the number of wingbeats, the number of glides, and the duration of each phase. The number of wingbeats in the flapping without glides was significantly higher than in the flapping with glides. The duration of each phase was also significantly longer in the flapping with glides than in the flapping without glides. The results indicate that the variable flapping pattern of the magpie has a significant effect on the respiratory pattern and mechanics of the bird.
24.3
FRONTAL ANALYSIS OF VENTILATORY CONTROL IN HETEROCHROMIC MAMMALS

During the reduced metabolic state of mammalian heterothermic torpor, the time course of ventilatory output shows that output and variability are accentuated compared with euthermia. The ventilatory responses to incremental hypoxia and hypercapnia were assessed in torpid bats of two species, Eptesicus fuscus and Pipistrellus hesperus, using whole-body plethysmography and computerized analysis. In general, hypercapnia elicits rhythmic, steady tidal volumes whereas hypoxia elicits irregular breath-to-breath intervals with mean tidal volume variation of up to 25%. Phase plots of ventilatory volume (V) vs. change in ventilatory volume (dV/dt) revealed a decreasing fractal dimension with increasing hypercapnia (1.4 for Pco2=38 torr) and an increasing fractal dimension with increasing hypoxia (1.8 for Pox2=30 torr) (p<0.0001 compared to hypercapnia). Breath-to-breath volume and intervals also both increased in fractal dimension from hypercapnia through hypoxia. However, the fractal dimensions never indicated purely random variation, thus implying that the lesser refined appearance of hypoxic-stimulated breathing may result from attenuated cooperation of control elements in a complex system. (Supported by NSF IBN-9200441)

24.4
NONLINEAR METHODS FOR THE ANALYSIS OF VENTILATORY CONTROL

Traditional measures of ventilatory response such as volume per time, frequency, inspiratory and expiratory times inadequately characterize patterns of instantaneous variation. For example, methods of Fourier analysis often used in frequency analysis assume underlying linear oscillators and thus cannot fully reveal the nonlinear dynamics of a multiple input system like the ventilatory controller. However, nonlinear analytical methods such as the fractal dimension provide a quantitative measure that can distinguish purely random variability from that indicative of an underlying complexity of control. Different methods for determining the fractal dimension may be used to advantage, each of which provide somewhat different values, but are useful as comparative measures between different ventilatory states and when compared to functions of known fractal dimension. Our investigations indicate ventilatory control to be a complex process displaying robust variability but deficient in pure randomness. (Supported by NSF grant IBN-9200441)

24.5
ACETYLCHOLINE STIMULATES BRAIN BLOOD FLOW RATE IN CRUCIAN CARP THROUGH A NITRIC OXIDE DEPENDENT MECHANISM
Goran E. Nilsson and Patrick Hylland. Vertebrate Physiology and Behaviour Unit, Dept. of Zoology, Uppsala University, Norbyvägen 20, S-752 36 Uppsala, SWEDEN

Nitric oxide (NO) dependent regulation of brain blood flow has not been proven to exist in fish. Using epifluorescence microscopy on the brain surface (optic lobes) of crucian carp (Carassius carassius), we here show that superfusing the brain with acetylcholine (ACh) induces an increase in cerebral blood flow rate that can be completely blocked by the NO synthase inhibitors NG-nitro-L-arginine methyl ester (L-NNAME) and NG-nitro-L-arginine. Also sodium nitroprusside, which decomposes to liberate NO, caused increase in cerebral blood flow rate. By contrast, L-NNAME could not block the increase in blood flow caused by anaesthesia. The results suggest that NO is a vasodilator in crucian carp brain that mediates the effects of ACh. Since teleost fish deviated from other vertebrates 400 million years ago, these results suggest that NO dependent brain blood flow regulation was an early event in vertebrate evolution.

24.6
CONTROL OF VENTILATION IN LOCUSTS. Jon E. Harrison, Phillip Wacławski*, Scotti Gullison*, and Katherine Kriplikis. Arizona State University, Tempe, AZ 85287

The importance, mechanism, and control of convective ventilation are all poorly understood in insects. In resting locusts, abdominal movements generate about 1 kPa min⁻¹. Convection increases CO₂ elimination by 50% by maintaining PCO₂'s in the air space consistently below hemolymph and muscle PCO₂'s. In resting locusts, ventilation rate (VR) is unaffected by variation in hemolymph pH, but is strongly affected by tracheal gases. Both decreases in tracheal PCO₂ and increases in tracheal PO₂ relative to normal values depress VR, demonstrating that quiescent locusts regulate internal gas levels rather than maximizing CO₂ excretion or O₂ uptake. Post-eclosion rises in VR are unaffected by manipulation of either hemolymph acid-base status or tracheal gas levels, suggesting that locomotion-associated rises in VR are caused by feed-forward and/or hormonal mechanisms. Funded by NSF IBN 9317784 to JH.

24.7
INTERACTION BETWEEN CO₂ RESPIRATORY DRIVE AND PULMONARY PRESSURE FEEDBACK IN BULLFROGS. Richard Kinkel and William K. Millson. Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4

In study we determined how the different components of the breathing pattern of the frog were affected by 1) 3 levels of respiratory drive (air, 1.7% CO₂, and 3.3% CO₂) with changes in phasic vs tonal lung pressure. Experiments were performed on decerebrate, paralyzed, unidirectionally ventilated frogs. "Fictive breathing" measurements were obtained by recording respiratory related motor nerve activity, showed that phasic and tonic changes in lung pressure had similar effects on breathing at all levels of CO₂. When respiratory drive was low, changes in lung pressure had little effect on "breathing" frequency or the spatio-temporal distribution of the breaths. Increasing inspired CO₂ levels increased breathing frequency, and the increase was progressively greater at greater lung pressures. In frogs ventilated with air and 1.7% CO₂, increasing lung pressure reduced the duration of the non-ventilatory period between breaths but did not affect the number of breaths in each episode. At the highest CO₂ level, increasing lung pressure also increased the number of breaths in each episode and the episodic breathing pattern virtually became continuous when lung pressure was elevated. Although the duration of the inspiratory phase of the fictive breath was shortened by increasing CO₂ and lung pressure, the duration of the total breath was only shortened by increasing CO₂. With lung deflation to 0 cm H₂O the fictive inspiration became equal to or greater than the period of glacial opening. We conclude that pulmonary stretch receptor and chemoreceptor feedback interact to produce the normal coordination of breathing movements and to enhance chemoreceptor responsiveness. Supported by NSERC and the Killiam trust.

24.8
EFFECT OF TIMING OF THE CO₂-RISE PROFILE ON VENTILATION IN GARTER SNAKES. Robert A. Fuller. Dept of Physiology, Univ. of Puerto Rico, School of Medicine. San Juan, PR 00936-5067

Garter snakes are sensitive to the rate at which intrapulmonary CO₂ rises during the non-ventilatory period. This information is used primarily to adjust breathing frequency. The present study was undertaken to determine whether snakes are equally sensitive to early or late rising lung CO₂. Snakes were unidirectionally ventilated with air and CO₂ from a gas mixer connected to a computer. The rate of rise of CO₂ was controlled by the computer which also monitored the snakes ventilation. On inspiration, CO₂ in the lung fell to zero, and at the end of inspiration, CO₂ began to rise according to the computer algorithm. The CO₂-rise profile was split into two phases, an early rise phase (0-2.5% CO₂) and a late rise phase (2.5-5%). When CO₂ rose slowly during both early and late rise phases (214 sec to reach 5% CO₂), breathing frequency was 0.28/min, and when CO₂ rose quickly during both phases (3.3 sec to reach 5% CO₂), breathing frequency was 4.23/min. If one of these rates were used for the first half of the CO₂-rise profile, and the other were used for the second half, an intermediate breathing frequency was adopted. When the early rise phase was fast, then slowed by 64-fold, breathing frequency was 1.92/min, and when the early rise phase was slow, then increased by 64-fold in the latter half, breathing frequency was 1.30/min. Other combinations were used with the same qualitative results. Therefore, snakes can sense a change in the rising CO₂ late in the non-ventilatory period and adjust breathing rhythm, and are probably adjusting rhythm continuously as CO₂ rises. Supported by NSF grant IBN 9316434.
THE PHYSIOLOGY OF BLOOD VOLUME REGULATION

25.1


Cardiovascular function is determined, in part, by the size and rate of circulating fluid spaces which have not been adequately measured in fish. Red blood cell space (RBC, $^{125}$I-RBC), albumin space ($\text{AS, } ^{125}$I-albumin), and extracellular space (ECS, $^{68}$Co-EDTA) were measured in 30 tissues of unanaesthetized rainbow trout, after circulation periods of 0.5, 1.2, 4.6, 16, & 24 hrs. ECS was larger (725-456 µL/g wet tissue) in kidney, swim blud, skin, fins; moderate (312-219 µL/g wet tissue) in gut, spleen, liver, intestine, gills, eyes, and caecum; and small (180-53 µL/g wet tissue) in red muscle, brain, gall bladder and white muscle. Of the 3 spaces, the ECS equilibrated the most rapidly (>70% equilibrium in all tissues except brain, eye, and gill blader in <1 hr). Equilibration of $^{125}$I-RBC was tissue specific and ranged from 100% equilibration in <0.5 hr (gills, brain, eye, red and white muscle, kidney, spleen, osocum, intestine, swimbladder, liver) to >24 hrs (fins, skin and skull). Similar results were noted with $^{125}$I-albumin, with the exception of red and white muscle, brain, eye, kidney, swimbladder and gill bladem which had not equilibrated in >24 hrs. A tissue-specific delay in the equilibration of $^{68}$Co-EDTA, particularly in red muscle (152 µL/g wet tissue), was noted. Two different compartments were defined: one with high ECS values was largely extracellular in nature (ECS), and the other with low ECS values was largely intracellular in nature (IC). The apparent extravasation of $^{125}$I-albumin suggests that it is a marker of vascular compartments in fish. Supported by NSF grant #DCB 9105247 to KO.

25.2

NATRIURETIC PEPTIDE RECEPTORS IN SHARK GILLS. John A. Donald*, Tse Tog* and David R. Evans. University of Florida, Gainesville, FL 32611, USA.

The presence of natriuretic peptides (NP) in the heart and brain of elasmobranch fish is well-established. Since all blood must pass through the gills before distribution to the body, the interaction between NPs secreted by the heart, and the gill tissues, could be critical in influencing NP function. The distribution and nature of natriuretic peptide receptors (NPR) in the gills of dogfish, Squalus acanthias were examined by tissue section autoradiography, kinetic and competition analysis, protein electrophoresis, and guanylate cyclase (GC) assays. Specific NP binding occurred on the gill filaments, but not on the interbranchial septum or gill arch. The binding was denser on the different edge of the gills. Higher resolution light-microscopic examination of sectioned shark gills showed specific binding occurring mainly on the secondary lamellae, and not the arterial circulation. At least two types of NPR were revealed: one is linked to GC since NP binding stimulates the production of cGMP; the other is a receptor with characteristics of the mammalian "clearance receptor" (NPR-C) since the specific ligand C-AMP (at two concentrations of NPR-C since the specific ligand C-AMP (at two concentrations 10(-10)-10(-6)M) displaced 50% of binding in sections and competition assays. The widespread distribution of NPR-C in the gills suggests that the plasma level of NP could be affected by changes in the perfusion profile of the gills which would effect the amount of receptor protein exposed to the blood. Supported by NSF DBS 9105161, NIHGM-F30-ESO3620.

95.3

EFFECTS OF GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR I ON SALINITY TOLERANCE IN THE ATLANTIC SALMON (Salmo salar). Stephen D. McCormick, S.O. Conte. Anadromous Fish Research Center, National Biological Survey, Turners Falls, MA, and Department of Biology, University of Massachusetts, Amherst, MA, USA.

The potential roles of growth hormone (GH) and insulin-like growth factor I (Igf-I) in seawater acclimation were examined in juvenile Atlantic salmon (Salmo salar). Both the short-term and long-term actions of these hormones were examined. Compared to controls, fish in 12 ppt seawater given a single injection of ovine GH (0.2 µg g⁻¹) followed 48 hours later by transfer to 34 ppt had significantly lower plasma sodium, osmolality and muscle moisture content. There was a similar, dose-dependent increase in salinity tolerance after a single injection of IGF-I (0.05-0.2 µg g⁻¹) followed 48 hours later by transfer to 25 ppt SW. GH and IGF-I did not increase gill Na⁺ K⁺-ATPase activity 48 hours after injection in either fresh water or 12 ppt. Fish in fresh water given GH implants (2.5-5.0 µg g⁻¹) for 4-10 days had greater gill Na⁺ K⁺-ATPase activity and salinity tolerance than controls. Cortisol implants (50 µg g⁻¹) also increased gill Na⁺ K⁺-ATPase activity and salinity tolerance, and in combination with GH had a synergistic effect. Although IGF-I implants (0.5-1.0 µg g⁻¹) alone for 4-10 days had no effect, cortisol implants were without effect. IGF-I and cortisol in combination increased gill Na⁺ K⁺-ATPase activity more than cortisol alone. The results indicate that IGF-I can carry out the short term but not the long-term actions of growth hormone on seawater acclimation and that both GH and IGF-I can interact with cortisol to increase gill Na⁺ K⁺-ATPase activity.
26.1 CHANGES IN TISSUE HEMOLYMPH AMINO ACID LEVELS IN RESPONSE TO AMMONIA-LOADING IN THE TERRESTRIAL ISOPOD PORCELLIO SCABER LART. Jonathan C. Wright, Sam Caveney, Michael J. O'Connell and Jennifer MacIsaac. Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1.

In an earlier study (J. exp. Biol. 188, 143-157) we reported the effects of ammonia-loading on ammonia (NH₃) excretion in the terrestrial isopod Porcellio scaber. Both ammonia and proline play a significant role in N-sequestration between periods of ammonia excretion. Here, analysis is extended to all major amino acids and to four digestive tract tissues (hepatopancreas, midgut, foregut and hindgut) and remaining somatic tissues ('body wall'). The pleopodal endopods are strongly implicated in outward transport of ammonia from the hemolymph and there are compartmentalized sites of ammonia ('ammonia-loading') for 7 days by exposure to a Fₐ₇ of 38 Pa generated by an appropriately buffered solution of (NH₄)₂SO₄. Concentrations were measured over a saturated, inorganic-free environment for the same period. Following this, tissues were dissected on ice, transferred to acetonitrile and centrifuged prior to AA analysis using reverse-phase HPLC. As AA's were determined in a saturated, inorganic-free environment concurrently with the tissue samples. Samples were spiked with hydroxyproline and a-amino butyric acid (AABA) as internal standards. The protein content for each of the tissues was determined using the Sigma 610 micro-determination method. Results were calculated as pmol AA/g hepatopancreas, hindgut, foregut and midgut respectively. Accumulations of glutamate, alanine and proline are also significant. Rate-limiting enzymes that catalyse these reactions cannot be metabolized to ammonia when humid conditions favor NH₃ volatilization.


Recent studies in our laboratory and others indicate that volume-activated (VA) release of organic osmolytes (amino acids, polypeptides and methyamines) occurs via a channel. We have shown that band 3 inhibitors such as DIDS inhibit this channel in skate (Raja erinacea) erythrocytes and have suggested that band 3 is involved in operation of the channel. However, most band 3 inhibitors also inhibit Cl⁻ channels, which are thought to be involved in organic osmolyte release in other cells. Therefore, we tested the effects of the band 3 inhibitor, pyridoxal-5-phosphate (P5P) (which does not inhibit Cl⁻ channels) on the VA channel in skate erythrocytes. Erythrocytes were incubated in isotonic (460 m0sm) elasmobranch Ringer with 0.1 mM P5P for 30 min at 15°C. The cells were isolated by centrifugation and assayed for radioactivity. Uptake rates of 3H-taurine, 3H-myo-inositol or 14C-betaine (46 mCi/ml) P5P (0.27±0.08) inhibited VA taurine, betaine and inositol uptake 5-15X via the same VA band 3 channel. The VA channel is regulated by the ratio of VA to Na⁺/H⁺ countertransport. P5P at a concentration of 2mM inhibited VA taurine and inositol uptake 90X±2% and 91X±2% respectively. These results provide further support for band 3 involvement in the operation of the VA channel.

PERSPECTIVES ON ENVIRONMENTAL PHYSIOLOGY

27.1 COMPARATIVE STUDIES OF THE Na⁺/H⁺ ANTIPORTER IN THE RED CELLS OF CARP, FLOUNDER AND TROUT. A. B. Cossins and F. R. Weaver. Department of Environmental and Evolutionary Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK.

Teleost red cells possess powerful Na⁺/H⁺ antporters which are activated by an energetically advantageous process in the trout, guppy and sturgeon. Hypotonic shrinkage in a nitrogen atmosphere caused activation of the B-NHE and a consequent regulatory volume increase response (RVI) in both flounder and carp red cells. Trout cells were completely unaffected by this treatment. RVI was also observed in carp cells held in a normoxic atmosphere but not in flounder red cells. Thus, fleounder B-NHE is shrink-activated and oxygenation-sensitive. By contrast, the carp B-NHE is shrink-activated but comparatively oxygenation-insensitive. Trout B-NHE is shrink-insensitive. Evidently, the antipporter responds to manifold stimuli, the exact responsiveness in any one species being of adaptive significance. The oxygenated uptake of K⁺ by the volume-activated B-NHE activity can be relieved by calcium A., a serine/threonine protein phosphatase inhibitor, and this activity can be 'clamped' by N-ethyl maleimide or 4-aminopyridine (AMP). The same two control pathways for volume-activation and oxygenation-sensitivity of the B-NHE both involve control of serine/threonine phosphorylation. (Supported by Wellcome Trust and N.E.R.C.)

27.3 MECHANISMS AND OXYGENATION DEPENDENCY OF VOLUME-ACTIVATED POTASSIUM AND AMINO ACID TRANSPORT IN CARP RED BLOOD CELLS. Frank R. Jensen. Institute of Biology, Odense University, DK-5230 Odense M, Denmark.

Hypotonic swelling of carp red blood cells (RBC's) induced a regulatory volume decrease (RVD). In a subsequent study, we restored the original cell volume within 140 min in oxygenated RBC's, whereas volume recovery was incomplete in deoxygenated RBC's. The complete RVD in oxygenated RBC's resulted from a sustained volume-activated release of K⁺, Cl⁻ and amino acids (AA). The contribution of inorganic and organic osmolytes to RVD was 70% and 30%, respectively. Oxygen per se activated a K⁺ efflux from RBC's. Hypotonic cell swelling stimulates an additional K⁺ release. The oxygenation-activated and the volume-activated K⁺ efflux were both inhibited by DIDS and by replacement of CO₂ by NO₂⁻ showing that both types of K⁺ release were CI⁻ dependent and probably via the same KCl cotransport mechanism. Once activated by oxygenation, the KCl cotransport was further stimulated by cell swelling. Deoxygination inactivated the oxygenation-induced CI⁻ dependent K⁺ release, and cell swelling was not a sufficient stimulus to significantly reactivate it. In deoxygenated RBC's, the volume-induced K⁺ release was transient and primarily CI⁻ dependent, and in the absence of ouabain the cell K⁺ content recovered towards control values via the Na⁺-K⁺ pump. Swelling-activated AA release differed in kinetics between oxygenated and deoxygenated RBC's but was important for RVD at hour 6 of the exposure. The AA release was about 70% inhibited by DIDS and 50% inhibited by replacement of CO₂ by NO₂⁻ suggesting that the AA permeation was partly CI⁻ dependent. The data communicate a significant influence of hemoglobin oxygenation on transport mechanisms involved in volume regulation of fish RBC's.
27.3
INTRASPECIFIC PHYSIOLOGICAL VARIABILITY AND HYPOXIA TOLERANCE AMONG INDIVIDUAL DOVER SOLE. Edward M. Godin*1, Eric A. Leroy* and Russell D. Vetem (SPON: G. Kooyman). NOAA, La Jolla, CA 92038; 1and Scripps Institution of Oceangraphy, Center for Marine Biotechnology and Richardson, U.C.-San Diego, La Jolla, CA 92033
We characterized the intraspecific variability of 15 respiratory and metabolic variables for the Dover sole, Microstomus pacificus, and related this variability to an individual's tolerance of hypoxia. These included blood oxygen affinity (P50), hematocrit, muscle ultra structure and lactate dehydrogenase activity, body length and condition, growth rate, relative gill size, muscle water content, and relative heart and spleen size. When exposed to declining oxygen concentrations over a five-day period, fish acclimated to hypoxia (98 weeks at 0.50 mg O2/l) survived to lower oxygen concentration (0.08 mg O2/l) than normoxic fish (0.22 mg O2/l). Multiple regression analysis indicated that these variables explained 73% of the intraspecific variability in order of death. Variables associated with the rate of energy use, e.g. enzyme activity, the rate of weight loss during starvation, and relative heart size, were not correlated with hypoxia tolerance. These data suggest that variability in oxygen uptake and delivery is more important for hypoxia survival than is variability in the rate of energy use. Results do not strongly support the concept of symmorphosis - as variables reflecting energy use, i.e. metabolic rate, were not highly correlated with an individual's capacity to deliver oxygen. (Supported by N.R.C., National Academy of Sciences)

27.4
Channel catfish were collected at eleven times during the period from Oct. 1991 to Jan. 1993 and acclimated in the laboratory to 7°C, 15°C, or 25°C for six weeks. Hepatosomatic index (HSI), mg protein/mg DNA, total liver DNA, total liver protein, and the activities of liver G6PDH, 6PGDH, and LDH were measured to examine seasonal variation in the acclimation response. Channel catfish collected in the fall and winter showed strong positive acclimation of HSI, mg protein/mg DNA, and total liver protein and DNA while spring and summer fish showed a reduced acclimation response. Activities of pentose phosphate pathway enzymes (G6PDH and 6PGDH) demonstrated positive acclimation in the fall and winter of the year; fish collected during the spring and summer demonstrated an inverse acclimation pattern. Liver LDH activity demonstrated little or no temperature compensation at any time of the year. All three enzymes showed positive acclimation when activity was expressed on a whole liver basis. Several important conclusions can be drawn from these experiments: (1) Enzymes of different metabolic pathways respond differently to temperature acclimation; (2) Changes in total protein and DNA play important roles in the acclimation response; the relative importance of each depends on acclimation temperature and season; (3) Enzymes from different metabolic pathways do not necessarily show the same seasonal variation in acclimation patterns; (4) Enzyme activities expressed on a whole liver basis may better represent in vivo acclimation than protein or DNA specific activities; (5) Seasonal differences in the acclimation response relate to the acclimatized state of the fish at the time of collection. The acclimatization state carries over into laboratory acclimation experiments and results in variable acclimation patterns.

27.5
INTRACELLULAR pH REGULATION IN COD RED BLOOD CELLS. Michael Beranbrink and Christian Rose. Institut für Zoologie, Lehrstuhl für Tierphysiologie, Heinrich-Heine-Universität, 40226 Düsseldorf, Germany
The red cell pH and pHe of Atlantic cod was studied in carbon dioxide / bicarbonate buffered media in the absence of catecholamines in February/March. At 10% CO2 (pHe 6.7) the transmembrane distribution ratio for protons was significantly reduced by 2.4-dinitrophenol, cyanide, low extracellular sodium, low extracellular chloride and by incubating the red cells in CO2-free media at similar low pHe. At 1% CO2, pHe 7.5 the anion transport inhibitor 4,4-diisothiocyanato stilbene-2,2'-disulfonic acid (DIDS) lead to a significant decrease of both pH and the intracellular sodium content. The results suggest that variability in oxygen uptake and delivery is more important for hypoxia survival than is variability in the rate of energy use. Results do not strongly support the concept of symmorphosis - as variables reflecting energy use, i.e. metabolic rate, were not highly correlated with an individual's capacity to deliver oxygen. (Supported by N.R.C., National Academy of Sciences)

28.1
Cryptocyanin, a newly described protein sometimes present in high concentrations in the hemolymph of the Dungeness crab, Cancer magister, is structurally similar to the oxygen transporting protein, hemocyanin. Cryptocyanin does not combine reversibly with oxygen, however. Hemolymph concentrations of cryptocyanin, 25S two hexameric hemocyanin and 15S hexameric hemocyanin were quantitatively monitored by pH 7.4 PAGE through the molt cycles of juvenile crabs. Levels of all three proteins were found to fluctuate in correlation to the molt cycle. Both hemocyanin remained low longer than did hemocyanin levels, since intact tripod hemocyanins are incorporated into exoskeleton. Different crab tissues were examined by pH 7.4 PAGE and SDS PAGE for the presence of hemocyanin and/or cryptocyanin. Hypodermal tissue, which synthesizes new exoskeleton, contained more cryptocyanin shortly after the molt than did other tissues. Western blots, using antibodies against C. magister cryptocyanin and hemocyanin, suggest that both proteins, especially cryptocyanin, are incorporated into the new exoskeleton. Supported by NSF IBN-9217530.

28.2
FRUIT LIPID COMPOSITION AND ITS RELATIONSHIP TO PREFERENCE AND ASSIMILATION IN FRUGIVOROUS PASSERINES. J. G. Groves and Edmund W. Stiles. Center of Macroeontology, Baltimore, MD 21202. Rutgers University, Piscataway, NJ 08855.
Fruit migrate frugivorous birds rephrasing fat reserves in eastern temperate deciduous forests preferentially consume fruit species high in lipid content. We investigated the nutritional role of lipids in fruit selection by: (1) determining pulp lipid composition of high-lipid fruits using TLC- and GC-FID; and (2) conducting preference and assimilation/retention experiments with captive wood thrushes (Hylocichla m. muscelina) on high-lipid fruits. Results suggest that Atlantic cod red blood cells exhibit sodium-dependent chloride/bicarbonate exchange to regulate their pHe under hypercapnic conditions in winter.

28.3
BIOCHEMICAL ADAPTATION

28.4
FRUIT LIPID COMPOSITION AND ITS RELATIONSHIP TO PREFERENCE AND ASSIMILATION IN FRUGIVOROUS PASSERINES. J. G. Groves and Edmund W. Stiles. Center of Macroeontology, Baltimore, MD 21202. Rutgers University, Piscataway, NJ 08855.
Fruit migrate frugivorous birds rephrasing fat reserves in eastern temperate deciduous forests preferentially consume fruit species high in lipid content. We investigated the nutritional role of lipids in fruit selection by: (1) determining pulp lipid composition of high-lipid fruits using TLC- and GC-FID; and (2) conducting preference and assimilation/retention experiments with captive wood thrushes (Hylocichla m. muscelina) on synthetic fruit diets varying in lipid content. Pulp lipids of eight high-lipid (15-50% dwt) fruit species were mostly triacylglycerols (70-99%) and phosholipids (3-10%) composed almost entirely (>90%) of oleic (46-50%) and palmitic (9-12%) acid. In patred-fruit choice trials captive wood thrushes preferred synthetic diets containing primarily unsaturated (vs. saturated) triacylglycerols. Mean retention time of [14C]-labeled triacylglycerols was longer (300 min) in birds offered an intermediate-lipid (20% dwt) diet than when offered in either a low-(4%) or high-(50%)lipid diet (4 min). Moreover birds did not show significant differences in assimilation efficiency (>90%) of the radiolabel among the diets, suggesting thrushes achieve a higher rate of net energy gain on a high-lipid fruit diet.
28.3
EFFECT OF EXERCISE ON THE PLASMA FATTY ACIDS: DOES AEROBIC CAPACITY MAKE A DIFFERENCE? Grant McClintock, George Zwirgenblat, C. Richard Taylor, and Jean-Michel Weber, University of Ottawa, Ottawa, ON KIN 6N9, Canada; Concord Field Station, Harvard University, Cambridge MA 02128.

Individual non-esterified fatty acids (NEFA) concentrations were measured in trained dogs and goats (V0max dog > V0max goat = 2.2 l/min) during treadmill exercise at 40 and 60% V0max. Our goals were to determine: 1) whether particular NEFA are mobilized or used preferentially during locomotion, 2) if differences in diet or aerobic capacity can affect the pattern of NEFA mobilization. Important differences in individual NEFA concentrations between the two species can be attributed to differences in aerobic capacity (V0max). The more aerobic species (dog) had higher plasma NEFA concentrations for all but one NEFA when compared with the sedentary species (goat). Also, exercise caused a large increase in concentration of individual NEFA in dogs (with the largest increases seen in oleate (150% above resting values) and palmitate (60% increase)), but had no effect in the goats. Unlike all other NEFA, stearate was present in similar concentrations in the two species and did not increase during exercise, even in dogs. Differences in diet and digestion physiology probably account for these differences in NEFA composition between the two species, and this is reflected in the percent contributions of individual FA to total NEFA: in dogs oleate > palmitate > linoleate > stearate, while in goats oleate > palmitate > arachidonic > linoleate. Linoleate, a NEFA of plant origin, was only found in goat plasma (6% total NEFA), while dogs had palmitoleate (7% total NEFA). Plasma NEFA composition in goats did not reflect that of their diet due to pre-absorptive modification of unsaturated FA while in dogs there was a good correlation between dietary NEFA and plasma NEFA. The more aerobic species (dogs) are able to maintain a higher plasma NEFA concentration in part due to a higher epinephrine concentration and a higher binding capacity than goats (albumin concentration is the same in the two species (0.54 mg/ml)). Higher [NEFA]plasma, along with higher cardiac output (Q), allows dogs to maintain a higher NEFA delivery important for endurance locomotion.

28.4
CHANGES IN MIDGUT ION TRANSPORT AND METABOLISM DURING LARVAL-LARVAL MOLTING IN THE TOBACCO HORNWORM C. M. Gibelius* and M. E. Chamberlin. Ohio University, Athens, OH 45701.

The tobacco hornworm (Manduca sexta) undergoes four larval molts and at each of these molts new cells are added to the midgut. To determine if there are changes in ion transport during molting, the transepithelial potential (PD) was measured in the posterior midguts of feeding (pre-molt) and molting fourth instars. Larvae in the final stages of molting were identified by the presence of fifth instar mandibles visible through a clear fourth instar head capsule. In vivo PD across the posterior midgut wall of larval larvae was 23.5% lower than that in feeding larvae, indicating that active ion transport is inhibited during molting. Citrate synthase activity, an index of maximal aerobic capacity, was also lower in the midguts of molting larvae (19.8 ± 0.9 pmol/min/g, n = 6) than in feeding larvae (30.8 ± 1.6 pmol/min/g, n = 6). The drop in active ion transport during molting may be due to this diminished aerobic capacity, which normally provides energy for active ion transport. This decline in aerobic metabolism may be partially offset by an increase in anaerobic glycolysis. During molting, the maximal activity of phosphofructokinase increased 36% and the maximal activity of lactate dehydrogenase increased 62%.

28.5

The subcellular distributions of enzymes involved in carbohydrate, fatty acid, and ketone body metabolism were examined in the hepatopancreas of the terrestrial snail Cepaea nemoralis. Maximal enzyme activities suggest that ketone bodies are important energy substrates in C. nemoralis. Interestingly, the enzyme b-hydroxybutyrate dehydrogenase (B-HBDH), which co-converts the ketone bodies acetoacetate and b-hydroxybutyrate, is localized exclusively in the cytoplasm of the hepatopancreas cells. An analogous condition is seen in liver and kidney cells of ruminant mammals. B-HBDH activity in all other known instances exists bound to the inner mitochondrial membrane.

28.6

The physiological correlates to temperature adaptation were studied using closed-chamber respirometry, spectrophotometric enzyme assays of cytochrome c oxidase (CCO), and fatty acid analysis in thermally acclimated winter-active (Cambarus bartoni) and winter-quiet (Orconectes propinquus) crayfish. Winter-quiet crayfish show a significant upward translation in the rate-temperature (R-T) curve of VO2 following acclimation to 20 °C (CA) vs. acclimation to 20 °C (WA). Winter-quiet crayfish show a variation in the R-T curve for VO2. The VO2 for Orconectes are generally more temperature-sensitive in all ranges of experimental temperatures (ave Q10 = 2.0 for O. p; Q10 = 1.7 for C. b.), and WA Orconectes are more temperature sensitive than CA. The R-T curve of the membrane bound aerobic enzyme, CCO, is also shifted upward in CA Cambarus, but not in Orconectes. Temperature sensitivity, however, is much lower in winter-quiet crayfish (ave. Q10 = 1.3) vs winter-active crayfish (ave Q10 = 2.1). A mechanism for compensation in the activity of CCO may be the fatty acid composition of tightly bound phospholipid molecules. Differences in fatty acid composition are currently under study.

28.7
PERIODIC AROUSALS DURING HIBERNATION: A POTENTIAL ROLE FOR DIFFERENTIAL GENE EXPRESSION. Stephanie A. Trelogan and Sandra I. Martin, UCSD, Denver, CO 80220.

Hibernation in mammals is widely recognized as an adaptive strategy for energy conservation. During the hibernation season, hibernators undergo a series of extended bouts of deep torpor punctuated by periodic rewarmings. These arousals, which squander at least 70 percent of the potential energy savings of hibernation, seem pointless, unless rewarming is necessary either for the survival of the animal or for the process of hibernation itself. Using a subtractive hybridization strategy, our lab has isolated a number of liver genes which appear to be important for maintenance of hibernation in ground squirrels, suggesting that differential gene expression plays a critical role in hibernation. To address this hypothesis, we are currently employing the technique of differential display to identify liver genes which are induced during alternating arousal and normothermy. We expect that the identification of these genes will help us to answer the question of why hibernators must periodically arouse throughout the hibernation season. Additionally, this endeavor should result in the identification of genes whose products play important regulatory or adaptative roles in determining the hibernating phenotype.

Supported by ARO Grant DAAH03-92-G-0019 to SLM.

28.8
SEASONAL VARIATION IN GLOUCOGENESIS IN A MAMMALIAN HIBERNATOR (Spalcomus lateralis). James F. Staples and Peter W. Hochachka, Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4, Canada.

Seasonal hibernation in mammals is usually associated with fasting, and since blood glucose and liver glycogen stores decrease during a hibernation bout, glucose metabolism is crucial to replenish carbohydrate supplies required by some tissues. Integyrase is the main metabolic fuel during hibernation and arousal, and since there is evidence of "protein sparing" during hibernation, it was predicted that glycerol would be a preferred gluconeogenic substrate during hibernation and arousal. Heparocytes isolated from Spalcomus lateralis in deep hibernation, after arousal from deep hibernation, or in summer normothermy were used to assess this. Rates of glucose production from 10mM lactate/1mM pyruvate (1.2 ± 0.1, 0.2 ± 0.1, 0.5 ± 0.1 pmol glucose/min/g, wet wt.) and 10mM alanine (0.3 ± 0.1, 0.5 ± 0.1, 0.3 ± 0.1 pmol glucose/min/g, wet wt.) were not significantly different from summer normothermy (1.1 ± 0.1, 1.3 ± 0.1, 0.5 ± 0.1 pmol glucose/min/g, wet wt.) These differences were reflected by rates of cellular oxygen consumption with the different substrates, but there was no difference in apparent oxidative efficiency of gluconeogenesis between hibernation states.
28.9 MITOCHONDRIAL FUNCTION IN MAMMALIAN LIVER DURING HIBERNATION. Hilary K. Serre and Sandra L. Martin*. UCHSC. Denver, Colorado 80262.

Mammalian hibernation is a unique physiological state in which the organism is able to sustain life at body temperatures as low as -3°C, without harming body tissues. The molecular processes involved in enabling an organism to maintain internal homeostasis at these low body temperatures have not been studied extensively. The liver is important in maintaining homeostasis during nonthermoregulation; thus, it is likely that liver mitochondria is important for survival of the organism during hibernation. In order to study liver gene expression and function during hibernation, we have isolated and partially characterized liver genes whose expression changes during hibernation in ground squirrels using a subtractive hybridization strategy. A subset of these genes have been identified as mitochondrial enzymes. We believe that during hibernation there is an up-regulation of mitochondria in the liver of ground squirrels. To test this hypothesis we are following two lines of experimentation: 1) ultrastructural and morphometric analysis of liver mitochondria in hibernating and active animals; 2) biochemical analysis of liver mitochondria from hibernating and active animals. Preliminary comparative studies of mitochondria isolated from hibernating and active ground squirrels show a loss of regulation in the oxidative-phosphorylation pathway in the mitochondria from the hibernating animals.

Supported by ARC Grant DAA-03-3-O-019 to S.L.M.


Cellulose acetate electrophoresis reveals the presence of two isozymes of arginine kinase in the posterior midgut of the tobacco hornworm (Manduca sexta). The activity of arginine kinase in homogenates of the midgut is 530 μmol/min/mg protein (n = 3). The activity in isolated midgut mitochondria is 540 μmol/min/mg protein (n = 3). It is unlikely that the presence of mitochondrial arginine kinase is due to cytosolic contamination because the procedure used to isolate mitochondria results in the isolation of mitochondria that are 95% pure. When supplementing the medium containing 0.1 mM palmitoyl carnitine, 0.05 mM malate, 5.0 mM MgCl₂, and 0.6 mM ATP, mitochondria require a rate of 15.9 ± 0.9 nmol O₂/min/mg protein (n = 5). This rate increases to 82.6 ± 5.2 nmol O₂/min/mg protein upon addition of 20 μM arsenate (n = 5). Arginine, itself, is not oxidized by these mitochondria. The arginine-stimulated respiration is inhibited by 0.01 M isatocyclid, indicating that the arginine kinase is probably located in the intermembrane space. This work was supported by an Ohio University Research Challenge Grant and funds from the Ohio University College of Arts and Sciences, College of Osteopathic Medicine, and Molecular and Cellular Biology Program.


Rates of evaporative water loss of insects have been correlated with cuticular lipids in many insects which have been studied on numerous occasions. The mechanistic basis for this relationship is hypothesized to be that cuticular permeability is determined by the physical properties of the lipid barrier, which are in turn determined by the stability of the surface lipid phase. As we move further along one link in this chain, i.e., how compositional changes affect lipid phase behavior, I examined the physical properties of pure palmitic acids (PAs) and simple HC mixtures, as a model for more complex natural mixtures. Melting temperatures (Tₘ) increased with chain length by ~2°C per carbon atom. Distearoylphosphatidylethanolamine branched and unsaturated decreased Tₘ by as much as 50°C, with internal substitutions having the greatest effects. HC mixtures mixed at intermediate temperatures, and the phase transitions were broader. Fractionation of cuticular HCIs isolated from several species corroborated the model mixture results. The order of decreasing Tₘ was: saturated (S) > branched (B) > unsaturated, and the Tₘ of total cuticular lipids was close to the weighted average of the melting points of the components. Supported by NSF grant IBN-9317471.


Cell membranes are one site of cold-induced injury. Some species, such as the goldenrod gall fly, Eurosta solidaginis, are tolerant of freezing. Little is known of the adaptations in the cell membranes of these species. We analyzed the fatty acids of larval Eurosta, as well as from another Dipteran that is freeze-susceptible, the fleshfly, Sarcophaga crassipalpis, and the larva of a Hymenoptera that parasitizes the Eurosta gall but is freeze-susceptible, Eurytoma gigantea. The fatty acids of the phospholipid (membrane) fraction of the two flies were more similar than the two freeze-susceptible insects, even though their overwintering strategies differed. Both flies were rich in (≥ 50%) in monoenes, mostly 18:1, as opposed to the wasp with ~27% monoenes, mostly 18:1. The wasp is rich in polyunsaturates (PUFA), comprising ~39% of fatty acids, about 2:3 of which were 18-carbon PUFA, and 1:3 20-carbon PUFA. The Diptera were PUFA-poor, with ~15% PUFA in Eurosta (all 18-carbons), and ~18% in Sarcophaga (~13% 18-carbons and 5% 20-carbon PUFA). The overall unsaturation, however, was greatest in Eurosta, with a unsaturation-to-saturation ratio of 3.22 as opposed to 2.36 in Sarcophaga and 2.28 in Eurytoma.

28.14 EXAMINATION OF CHOLESTEROL'S FUNCTIONAL ROLE(S) IN THERMAL ADAPTATION OF BASOLATERAL MEMBRANES FROM RAINBOW TROUT. Elizabeth L. Crockett and Jeffrey R. Hazl. Department of Zoology, Arizona State University, Tempe, AZ 85287.

Previously we have found that cholesterol content does not change with acclimation temperature in intestinal basolateral membranes (BLM) from rainbow trout. Although these membranes exhibit perfect homeoviscous adaptation, cholesterol would not appear to be involved in this adaptation. Cholesterol, however, may play a role in preserving functionality of membrane-associated proteins. Yeagle and colleagues (1988) have shown with mammalian plasma membranes that there exists an optimal level of cholesterol needed for membrane function. We have isolated and examined basolateral membranes from trout intestinal epithelia by determining how relative cholesterol levels affect the activity of Na⁺K⁺ATPase. We predict that when measured at physiological temperatures, we will see a loss of regulation in the oxidative-phosphorylation pathway in the mitochondria from the hibernating animals.

Supported by NSF grant IBN-9106652.


The effects of oxygen limitation and extramitochondrial pH on mitochondrial protein synthesis were investigated in isolated mitochondria from encysted gastrulae of Artemia franciscana. In intact embryos, an anoxia-induced acidification of intracellular pH (pHₐ) is thought to promote the arrest of both anaerobic and catabolic processes in the cytoplasm. Elucidating the variables responsible for down-regulation of these processes in the mitochondrion would provide a more complete understanding of whole-cell physiology during quiescence. At the optimal pH of 7.5, exposure of mitochondria to anoxia resulted in a 79% reduction in the rate of protein synthesis in vitro. Lowering pH to 6.8 (the pH observed under anoxic in vivo) suppressed protein synthesis by an additional 10%. Taken together, these data indicate a direct role for oxygen depletion in the suppression of mitochondrial protein synthesis. No differences in translation products were detectable as a function of oxygen tension or pH. Extramitochondrial ATP levels did not change after 1 h of anoxia or as a function of extramitochondrial pH, while ATP levels decreased by up to 35% in response to anoxia. Thus, changes in protein synthesis rates are unlikely to be the result of changes in at least the proximal energy source. Measurements of intramitochondrial pH using the fluorescent probe 2',7'-biocarboxyethyl-5(6)-carboxyfluorescein (BCEF) show that pHₐ is maintained for at least 1 h of anoxia. In summary, oxygen limitation and, to a lesser extent, acidic extramitochondrial pH likely contribute to the arrest of mitochondrial protein synthesis during anoxia. Measurements of the rate of mitochondrial protein synthesis as a function of endoplasmic protein, both in the presence and absence of initiation or elongation inhibitors, are currently being conducted. Supported by NSF grant IBN-9106652.


Cellulose acetate electrophoresis reveals the presence of two isozymes of arginine kinase in the posterior midgut of the tobacco hornworm (Manduca sexta). The activity of arginine kinase in homogenates of the midgut is 530 μmol/min/mg protein (n = 3). The activity in isolated midgut mitochondria is 540 μmol/min/mg protein (n = 3). It is unlikely that the presence of mitochondrial arginine kinase is due to cytosolic contamination because the procedure used to isolate mitochondria results in the isolation of mitochondria that are 95% pure. When supplementing the medium containing 0.1 mM palmitoyl carnitine, 0.05 mM malate, 5.0 mM MgCl₂, and 0.6 mM ATP, mitochondria require a rate of 15.9 ± 0.9 nmol O₂/min/mg protein (n = 5). This rate increases to 82.6 ± 5.2 nmol O₂/min/mg protein upon addition of 20 μM arsenate (n = 5). Arginine, itself, is not oxidized by these mitochondria. The arginine-stimulated respiration is inhibited by 0.01 M isatocyclid, indicating that the arginine kinase is probably located in the intermembrane space. This work was supported by an Ohio University Research Challenge Grant and funds from the Ohio University College of Arts and Sciences, College of Osteopathic Medicine, and Molecular and Cellular Biology Program.

Supported by NSF grant IBN-9317471.
28.15 TEMPERATURE ACCLIMATION ALTERS THE PHASE BEHAVIOR OF TROUT LIVER PLASMA MEMBRANES. Jeffrey K. Hazel and Susan J. McKinley, Department of Zoology, Arizona State University, Tempe, AZ 85287.

Plasma membrane microdomains enriched in canalicular membranes were isolated from liver tissue of rainbow trout (Oncorhynchus mykiss) acclimated to 5 and 20°C by a combination of differential and Percoll gradient-density centrifugation. The fluorescent probes laurdan (6-carboxy-1,3,5,7-tetrakis(2-methyl-4-phenyl-6-phthali-

28.16 MEMBRANE ADAPTATION TO SOLUTE SYSTEMS IN A. Ballantraea and H.C. Green. Department of Zoology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

The phospholipids and phospholipid fatty acid composition of mitochondrial membranes of organisms using different solute systems were compared. The organisms examined include the oyster, Crassostrea virginica, the hagfish, Myxine glutinosa, the elasmobranch, little skate, Raja erinacea, and the marine teleost, the winter flounder, Pseudosciaena crocea. Where possible, the membrane temperature and salinity and fed the same diet (hagfish, elasmobranch, winter flounder). Salinity acclimation in the osmoconforming oyster results in increases in the content of anionic phospholipids of all mitochondria at high salinities consistent with a strategy to counteract the effects of increasing intracellular levels of inorganic ions. Comparison of the mitochondrial lipid of liver mitochondria of three species of marine fish using different osmotic strategies (hagfish, skate and winter flounder) indicate no differences in the phospholipid composition but profoundly different phospholipid fatty acids in the elasmobranch. The fatty acids in the elasmobranch phospholipids were much more saturated that those of the other two species. This is consistent with adaptation to the perturbing effects of the fast fluctuations of ionic strength in elasmobranch tissues.

The results suggest mitochondrial membrane adaptation to altered intracellular inorganic ions as well as to naturally occurring chaotropic agents such as urea.

28.17 SEASONAL AND DIURNAL COMPARISON OF HSP70 LEVELS IN THE MUSSEL MYTILUS CALIFORNIIANUS COLLECTED FROM DIFFERENT HEIGHTS IN THE INTERTIDAL ZONE. Deirdre Stearns, University of Colorado, Boulder, CO 80309.

The objective of this study was to quantify levels of HSP70 stress proteins in the intertidal mussel Mytilus californianus from different osmotic strategies (hagfish, skate and winter flounder) indicate no differences in the phospholipid composition but profoundly different phospholipid fatty acids in the elasmobranch. The fatty acids in the elasmobranch phospholipids were much more saturated that those of the other two species. This is consistent with adaptation to the perturbing effects of the fast fluctuations of ionic strength in elasmobranch tissues.

During unfavorable environmental conditions, embryos of the brine shrimp Artemia franciscana enter a state of quiescence that can last for several months. Many key cellular functions including protein synthesis are suppressed. If degradation of protein were to continue in the cell, metabolic machinery required for recovery from quiescence could eventually be degraded. Hence, inhibition of protein synthesis is potentially crucial. An in vitro assay for proteolysis was developed with Artemia lysates, which rely on the libera-


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Post-diapausing gemmules of the freshwater sponge Funiculus fragilis undergo a marked increase in metabolism during germination. The increased metabolism is at least partly due to an increased consumption of sorbitol. Sorbitol levels decrease from 0.5 to approximately 0.75 mg/gfw during germination. Protein and lipid levels remain unchanged. Glycogen levels increase from 3.8 to a maximum of approximately 15 mg/gfw at 22 hours and then decrease to a steady state level of 14 mg/gfw. The breakdown of sorbitol results from an increase in the activity of sorbitol dehydrogenase from undetectable levels in dormant gemmules to a maximum of 0.22 umoles/mg protein after 30 hours of exposure to 4°C. Addition of glucose and sorbitol during germination increased the rate of sorbitol breakdown. The pattern of sorbitol breakdown at 22 hours in sorbitol dehydrogenase activity remains constant throughout germination. The activity of glycogen synthetase does not change during germination; however, the activity of glucose 6-phosphate dehydrogenase is approximately 15 times greater than the activity of glucose 6-phosphate-dependent glycogen synthetase. Although pure speculation at this time, it is possible that an increase in glucose-6-phosphate concentration could result in an increase in the synthesis of glycogen. Total glycogen phosphorylase activity increases from about 0.0015 umoles/min/mg protein to 0.0035 umoles/min/mg protein during germination. At the same time, however, the percentage of glycogen phosphorylase catalyzes a decrease from almost 100% to about 80%. This would stimulate the apparent increase in activity. The role of other effects of glycogen phosphorylase activity remains to be examined.


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28.21
SCALING OF THE PHOSPHORYLATION RATIO WITH BODY MAGG;
ARE THERE THERMODYNAMIC LIMITS TO MAMMALIAN BODY SIZE?
Geoffrey P. Dobson, and John P. Head. Department of Molecular Sciences, James Cook University of North Queensland, Townsville, Qld. Australia 4811

Scaling of body metabolism has intrigued scientists for many centuries. The cytosolic phosphorylation ratio (ATP/ADP.Pi, M⁻¹) in the rat, rabbit, dog and human heart was found to be inversely related to body mass with a regression line of slope of -0.30 (R=0.999). This exponent is similar to that reported for relationships between specific metabolic rate (e.g. oxygen consumption) of mammals. The Gibbs energy of ATP hydrolysis, (AG(ΔG)) the thermodynamic efficiency of ATP production (energy captured in forming three ATP along the mitochondrial respiratory chain from NADH to 1/2 O₂), mechanical efficiency and the inverse of true cytosolic [ATP] of the myocardium were all found to scale in the same direction as mass specific metabolic rate and body mass. These parameters provide new insights into the kinetic control of flux rates in small versus large mammals. Furthermore, we conclude that smaller mammals are more efficient in converting energy from the oxidation of foodstuffs to the bond energy of ATP compared to large mammals, and that the lower limit for an adult 2.0 g endotherm (e.g. bubble-free bat, Estrous shrew and hummingbird) may be set by the thermodynamics of the mitochondrial electron transport chain.

28.22

Previous studies have documented that blood glucose concentrations of turtles rise during periods of anoxia. This study measured glucose uptake by sciotic and cardiac muscle to determine if cardiac muscle had preferred access to blood glucose levels during anoxia. Uptake was measured using strips of skeletal muscle (pectoralis major) and ventricular tissue, equilibrated in 5 or 10 mM glucose with 1 uCi/mg O-[14C]glucose, a non-metabolizable analog of glucose added as a radiolabeled tracer. Samples were removed at 10 minute time intervals over 90 minutes. For anoxia studies, tissues were submerged in N₂ equilibrated water (pO₂ < 3 torr) for 14 hours, then dissected and glucose uptake measured in O₂ free conditions. There was no significant difference in glucose uptake between sciotic and cardiac muscle under either normoxic or anoxic conditions. However, glucose uptake was significantly reduced during anoxia in both skeletal and cardiac tissues (95 vs 40 mmol/gm h for control vs anoxia). Concerning that metabolic rate of turtles is reduced ten-fold during anoxia, the rate of glucose uptake was significantly higher during anoxia in both tissues. We conclude that anoxic muscle tissue has a higher rate of glucose uptake when corrected for metabolic rate but that cardiac tissue does not have preferred access to glucose.

This work was supported by NHLBI grants (C.L. Watson and W.L. Few) and an NSF grant (D.C. Jackson).

28.23

Uptake of 2H-2-deoxyglucose into scimitar tendons (SEMI) and vastus lateralis (VL) of goats provided indices of glucose utilization (GUI) in hindlimb muscles of resting and running (60% of VM-max) animals. At rest, GUI in SEMI was about 3-fold higher than that of the VL. However, exercise resulted in a 50-fold increase in the GUI of VL, while GUI in SEMI increased by only 4-fold. Together, the SEMI and VL comprise about 2% of the total body musculature of goats and at rest these tissues accounted for about 2% of the whole-body glucose disposal. During exercise, when plasma glucose concentration doubled and glucose disappearance rate increased by 3-4 fold, SEMI and VL accounted for 10-15% of whole-body glucose disposal. Respiratory quotients of 0.9-1.2 in running goats indicate dependence on carbohydrate-based fuels. The exercise-induced increase in the proportion of whole-body glucose that is disposed of in hindlimb muscles is consistent with the generalization that circulating glucose is a primary component of total carbohydrate oxidation in animals that are not highly aerobically adapted.

29.1

In grasshoppers, hindlimb autotomy severs the nerve to the leg and, transnurally, induces atrophy of metamorphic muscles actively damaged or denervated by autotomy. Extent of atrophy varies with development and across species. Muscles atrophy severely following autotomy at any age in B. atrocarus, a bundle of neurons and their targets, and how they change in development and evolution. This system is useful for the study of regulatory interactions among neurons and their targets, and how they change in development and evolution. Following autotomy, the nerve fibers atrophy dichotomously, both within a single muscle and among muscles. In B. poioa, a bundle of fibers in the posterior of muscle #120 (m. #120) exhibits pronounced resistance to atrophy, while anterior fibers degenerate completely. Our studies reveal differences between the anterior and posterior fibers of m. #120: 1) proctolin-like immunoreactivity is associated exclusively with posterior fibers. HPLC analysis of the muscle extract and bioassay of the fraction co-eluting with authentic proctolin support the presence of this peptide on the muscle. 2) Histochomical fiber-typing reveals thin fibers of the slow, oxidative fiber type in the posterior bundle. In contrast, the remaining larger-diameter fibers are of the fast/intermediate type. Hale and Burrows (1985) reported the innervation of several metamorphic muscles by the common inhibitor motorneurones (CI), including the posterior fibers of m. #120. We compared the extent of atrophy in muscles of this pool and found no such correlation with innervation by the CI. We are continuing to investigate the association of these properties with the degree of atrophy in m. #120 and other muscles in B. poioa, as well as in juvenile and adult S. americana and M. differentialis. (Supported by NIH T32 HD07396 and NSF IIB 9210394).

29.2
ANOXIA REDUCES N-METHYL-D-ASPARTATE RECEPTOR ACTIVITY IN FRESHWATER TURTLE CEREBRAL CORTEX. Phillip E. Bickler and Leslie T. Buck. Dep't of Anesthesiology, University of California, San Francisco, CA 94143-0242

Freshwater turtles survive prolonged anoxia with no brain injury. A key aspect of this adaptation may be down-regulation of excitatory neurotransmission, and avoidance of attendant energy expenditure and excess calcium influx into post-synaptic neurons. We previously showed (JJP 225: R277-R281) that glutamate-mediated calcium influx was 75% suppressed by anoxia, but we did not determine the receptor subtype(s) responsible. We therefore tested whether the N-methyl-D-aspartate subtype glutamate receptor, which mediates excitatory neurotransmission in brain cortex, is inactivated during anoxia.

Functional NMDA receptor activity was measured as NMDA-induced calcium influx (fluorescence) in brain cortex sheets from Chrysemys picta. In brain sheets from normoxic turtles, 50 μM NMDA increased cytosolic calcium ([Ca²⁺]c) by 497 nM. After 14-18 hours of anoxia, this was reduced to 341 nM (~4.05, unpaired t-test), Anoxia reduced ten-fold during anoxia, the rate of glucose uptake was significantly higher during anoxia in both tissues. We conclude that anoxic muscle tissue has a higher rate of glucose uptake when corrected for metabolic rate but that cardiac muscle does not have preferred access to glucose.

This work was supported by NHLBI grants (C.L. Watson and W.L. Few) and an NSF grant (D.C. Jackson).

COMPARATIVE NEUROBIOLOGY
ROLE OF ADENOSINE IN N-METHYL-D-ASPARTATE RECEPTOR MODULATION IN AN ANOXIA TOLERANT TURTLE (CHRYSES'TYS PICTA BELLII). Leslie T. Buck and Philip E. Bickler. Dept. of Anesthesia, University of California, San Diego, CA 92103-0527.

Accumulation of the neurotransmitter adenosine (AD) in the anoxia tolerant turtle brain may play a key in a protective decrease in excitatory neurotransmission during anoxia. To determine whether AD decreases NMDA (N-methyl-D-aspartate) receptor activity in the turtle, we measured NMDA-mediated calcium influx in cortical cells using the Ca²⁺ sensitive dye fura-2. Adenosine decreased Ca²⁺ influx via the NMDA receptor from a control of 287 nM to 103 nM (64%). This effect is mediated via the A₁ receptor since 8-phenylthophylline (specific A₁ antagonist) effectively blocked the AD effect, and 8-phenylxanthine (A₂ antagonist) elicited a similar decrease in the NMDA mediated Ca²⁺ influx (44%). Cortical sheet ATP was maintained throughout the protocol and after single and multiple NMDA exposures at levels toxic to mammalian neurons. Preliminary whole-cell attached-patch clamp recordings of NMDA mediated Ca²⁺ currents in turtle cortical slices indicate that part of the adenosine effect is a membrane potential dependent. We speculate that adenosine causes hyperpolarization of the postsynaptic membrane and this strengthens the voltage-dependent Mg²⁺ block of the NMDA receptor, reducing its activity. Therefore, adenosine leads to a functional reduction in NMDA receptor activity in turtle cerebrocortex and may contribute to the extreme anoxia tolerance of the turtle brain. Supported by MRC Canada and NIH GM52215.

CORTICAL OSCILLATIONS IN TURTLE CORRELATE WITH SPONTANEOUS VISUAL BEHAVIORS. James C. Prechtl* and Theodore Rullo. Neurobiology Unit, Scripps Research Institute, La Jolla, CA 92037.

Visual stimuli such as light flashes and moving bars evoke 20 Hz spindle-like oscillations in the dorsal cortex of the turtle with and without associated oculomotor responses. The 20 Hz spindles share a number of features with cortical oscillations (ca. 35-70 Hz) observed in mammals during sensory processing. In this study we examined for similar 20 Hz spindles during spontaneous oculomotor behaviors in an unanesthetized environmental setting.

Epipial arrays of 5 electrodes each were placed symmetrically on left and right dorsal cortices of locally anesthetized, partially restrained turtles (Podocnemis unifilis). Field potentials were band passed between 1-100 Hz and recorded continuously for 15 min periods. Oculomotor responses were analyzed with concurrent electro-oculograms and close-up infrared video.

In the static visual environment, shifts of gaze occur at a rate of 2-3 per min, and most (>70%) involve both eyes leading by tens of milliseconds. All discernible eye movements were associated with cortical spindles although 30% of observed spindles occurred without eye movements. Movement related spindles were bilateral and lasted 1-7 sec. The increases in 20 Hz activity began with or slightly before the movement of the leading eye. Spindles contralateral to the leading eye contained greater power in the 70 Hz band and their peak amplitudes were usually between 100 and 300 micro Volts, greater than ipsilateral spindles. Changes or small oscillations in pupil diameter occurred at the completion of most eye movements. Spindles changes without eye movements also occurred and were associated with similarly lateralized 20 Hz spindles. Eye lid opening evoking similar spindles of large amplitude that lasted 1-2.2 sec in duration. These and previous results (EEG Clin. Neurophysiol. in press) indicate that 20 Hz spindles in the turtle's visual cortex occur conditions when visual input is actively or passively changed.
39.1 CLONING AND EXPRESSION OF SALMONID CARDIAC TROPONIN C: ROLE IN TEMPERATURE SENSITIVITY OF CARDIAC MYOFIBRILS. C. D. Moyes, T.J. Borgford, L.L. Blance, G.F. Tibbitts, Simon Fraser Univ., Burnaby, BC, V5A 1S6, Canada

The relationship between intracellular Ca^2+ sensitivity, temperature and pH exhibited by salmonid cardiac myofibrils differs in several aspects from that of mammals. We investigated the role of the myofibrillar Ca^2+ binding protein, tropinin C (TnC), in determining these properties. Cardiac TnC has 3 functional Ca^2+ binding sites, being located at the regulatory site (cII) and two at the troponin complex (cI and cII). Steady state occupancy of these sites at various temperatures was determined. Cardiac TnC was produced (FW27-cTnC) by site-directed mutagenesis (single stranded oligo) which increases the ability of the protein to bind Ca^2+ better at lower temperatures. The relative affinities of cII and cI at physiological temperatures were determined in vitro and in situ. The temperature-dependent changes in the relative affinities of the regulatory sites are consistent with changes in Ca^2+ sensitivity. These results suggest that the primary structure of salmonid cardiac TnC is different from that of mammals, and that it may play an important role in determining Ca^2+ sensitivity in these species.

39.2 TEMPERATURE SENSITIVITY OF SALMONID MYOCARDIAL Ca^2+ SENSITIVITY. C. Chaturvedi, C.D.Moyes, G.F. Tibbitts, Simon Fraser Univ., Burnaby, V5A 1S6, Canada

Mammalian heart demonstrates marked decrease in Ca^2+ sensitivity and isometric tension in response to decreased temperatures. In salmonid hearts exhibited Ca^2+ sensitivity that decreases at low temperature (9°C) by 15% in vivo. The Ca^2+ sensitivity is more temperature dependent (9°C to 0°C) by 30% as a result of endothermic myocardial contractility at temperatures that are cardioplegic for mammals. We postulated that the mechanism is in myofibrillar Ca^2+ sensitivity (Kc,a) or its temperature dependence (delta Kc,a/ delta T) which increases in vivo at colder temperature. As Kc,a is also determined by intracellular pH (pHc,a), we determined the regulation of Ca^2+ sensitivity by intracellular pH in vivo. We observed a significant increase in Ca^2+ sensitivity in fish that were exposed to lower temperatures. These results suggest that the primary site for intracellular pH regulation is intracellular pH. Further, the potential role for intracellular pH in vivo has been demonstrated in salmonid hearts. The oxygen saturation of intracellular pH (pHc,a) would be sufficient to overcome the effect of temperature on Ca^2+ sensitivity.

39.3 IS ONE PERFORMANCE MEASUREMENT ENOUGH? ALTERNATIVES TO U<_T METHODS FOR EVALUATING MUSCLE PERFORMANCE. Jay A. Nelson, Shannon Reid, Dale Webber and Steve Kerr, Dalhousie Univ., Halifax, N.S., Canada 1HR 4J1

Despite its use, the U<_T method of evaluating muscle performance in fish has limitations. Only one performance measurement is used to estimate the maximum swimming speed (U<_T) of fish. The U<_T test involves maximum swimming speeds of fish, which are then compared with the laboratory swimming speeds to estimate the U<_T of the fish. However, the U<_T test has limitations, such as the difficulty in determining the maximum swimming speed of fish. The U<_T test also involves a single measurement of muscle performance, which may not be representative of the overall performance of the fish. Other methods, such as the laboratory swimming tests, may provide more accurate estimates of muscle performance. Therefore, it is important to consider alternative methods for evaluating muscle performance in fish, such as the laboratory swimming tests, when evaluating muscle performance in fish.

39.4 ENVIROMENTAL DETERMINANTS OF WHITE MUSCLE PERFORMANCE: LESSONS FROM THE DEEP-SEA. B. J. L. Chen and G. A. Gravley, NOAA Fisheries, NMFS, South Carolina Cooperative Institute, Charleston, SC 29412-2311.

Environmental conditions, such as temperature and salinity, can have a significant impact on the performance of white muscle in deep-sea species. The relationship between white muscle performance and environmental conditions can provide insights into the physiological mechanisms underlying white muscle performance in deep-sea species. Understanding these relationships is crucial for the conservation and management of deep-sea species. Further research is needed to investigate the effects of environmental conditions on white muscle performance in deep-sea species, as well as the potential implications for conservation and management strategies.
39.7 MORPHOLOGICAL AND ENZYMATIC CORRELATES OF BURST SWIMMING SPEED IN TREE FROG TADPOLES. By Timothy B. Watkins. Department of Ecology and Evolutionary Biology, University of California, Irvine CA 92717.

The functional determinants of burst locomotor performance in animals are poorly understood. Correlates between burst speed and morphological activity, muscle contractile properties or morphology among individuals are rarely tested. Typically enzymatic determinants are sought among activities of glycolytic enzymes; this might not be fruitful.


Exhaustive exercise in rainbow trout results in depletion of muscle glycogen stores and elevation of muscle and plasma lactate and plasma cortisol. During the first 2h after exercise plasma cortisol levels are elevated and there is no net glycogen re-synthesis. The exercise-induced rise in plasma cortisol was blocked by either treatment with metyprene (2-methyl-1,2-di-3-pyridyl-1-propanone), to block cortisol synthesis or dexamethasone, to inhibit cortisol release. Neither treatment affected exercise performance, but unexpectedly blocked the exercise-induced rise in plasma cortisol. In treated fish restoration of acid-base and metabolite status was complete within 2h, compared to 8h required in control fish. To determine whether this is a cortisol-specific effect, metyprene-treated fish were infused with cortisol for 2h post-exercise. Plasma cortisol levels in these fish were similar to those in controls, and the inhibition of metabolic recovery was restored: in cortisol-infused fish, the time required for lactate clearance was similar to that in control fish. Our working hypothesis is that cortisol exerts its effects through stimulation of corticosteroid receptors, and that the muscle glycogen phosphofructokinase system is involved.


A specific activity of an enzyme associated with rates of muscular growth in C. opilio. Glycolytic and mitochondrial enzyme activities as well as RNA/DNA ratios were measured in the muscle from walking legs. The effect of size and maturity on the enzymatic activity was first assessed. Mitochondrial enzyme activities were higher in mature males than in immature crabs and were affected by the size of the animals. Therefore, we chose mature males of similar carapace width to investigate relationships between growth and the selected variables. Three groups of animals in each size category were exposed to different food rations and were collected after 26 and 60 days of experimentation. Muscular tissue growth was highly correlated with the hepatosomatic index and the activities of cytochrome-C oxidase, citrate synthase and lactate dehydrogenase but independent of non-proteinuric activities and RNA/DNA ratios. The relationships differed considerably according to whether the enzyme activities were expressed per g of wet tissue or per mg of DNA. This research was supported by NSERC.

39.10 MUSCLE METABOLISM IN A DRAGONFLY THAT UNDERGOES AN ONTOGENETIC SHIFT IN THERMAL SENSITIVITY OF FLIGHT PERFORMANCE. Karen L. Dennis* and James H. Marden. Pennsylvania State University, University Park, PA 16802.

The dragonfly Libellula pulchella shows a novel developmental pattern of adult morphogenesis accompanied by a large-scale change in thermal sensitivity of flight performance. Newly emerged adults (teneral) show peak flight performance at muscle temperatures of 28-34°C, whereas mature adults show peak flight performance at 40-48°C. This is the first demonstration of an ontogenetic shift in thermal sensitivity of insect muscle performance, and we are interested in determining both the mechanistic basis and the evolutionary significance of this trait. Changes in muscle color (wings in teneral; red in mature) and ultrastructure (distribution of mitochondria in teneral) suggest that energy metabolism in L. pulchella flight muscle might shift from anaerobic to aerobic during adult maturation, and we hypothesized that this shift in energy metabolism might cause the ontogenetic change in thermal sensitivity of flight performance. Thus, we examined 1) activity and thermal sensitivity of anaerobic (lactate dehydrogenase) and aerobic (citrate synthase) enzymes, 2) accumulation of glycogen and lactate, 3) extracellular activity in L. pulchella dragonflies of various ages. LDI activity in nymphs was high (50-60 uM product/minute), decreased by about half in teneral, and in nym Was almost nonexistent. Citrate synthase activity increased from very low in nymphs to levels in excess of 60 uM product/minute in nym Was. However, glycogen content and lactate accumulation were consistently low for all developmental stages, and the rate of fatigue was indistinguishable between nymphal and mature insects. These results indicate that even though tenevals possess an unusually high level of LDH activity for an adult insect, they do not appear to utilize the full anaerobic pathway. Ontogenetic changes in energy metabolism do not appear to cause the shift in thermal sensitivity of adult flight performance. Supported by NSERC grant B-93-17969.

39.11 SURFACE-SKIMMING STONEFISHES. DEMONSTRATION OF A POSSIBLE INTERMEDIATE STAGE FOR THE EVOLUTION OF INSECT FLIGHT. James H. Marden and Melissa G. Zomer*.

Pennsylvania State University, University Park, PA 16802.

An accumulating body of fossil, neurological, and developmental evidence suggests that insect wings evolved from gill plates used originally by aquatic insects. Two major stages in the evolution of insect flight are the evolution of wings and swimming, yet the nature of intermediate stages in the evolution of flighters from swimmers remains a mystery. Here we describe a form of non-flying insect, the surface-skimmer, in which aquatic insects use their wings to push water for propulsion while remaining in contact with the surface of the water. Support for the notion that surface-skimmers may be ancestral forms of insects that evolved wings but have retained their aquatic mode of locomotion is provided by wing-flapping, while continuous contact with the water surface removes the need for total aerodynamic weight support. We measured surface-skimming velocity and its dependence on wing area, and muscle power output. Morphological features of muscles that appear to be specific adaptations for surface-skimming (wet-resistant hairs on the wings and venal tarsi) are shared with the primitive subadult stage of mayflies ("subimagos"; nymphs), and stoneflies (subimagos) could have simultaneously undergone directional selection for improvement in performance. Newly emerged adults (teneral) show peak flight performance at muscle temperatures of 28-34°C, whereas mature adults show peak flight performance at 40-48°C. This is the first demonstration of an ontogenetic shift in thermal sensitivity of insect muscle performance, and we are interested in determining both the mechanistic basis and the evolutionary significance of this trait. Changes in muscle color (wings in teneral; red in mature) and ultrastructure (distribution of mitochondria in mature) suggested that energy metabolism in L. pulchella flight muscle might shift from anaerobic to aerobic during adult maturation, and we hypothesized that this shift in energy metabolism might cause the ontogenetic change in thermal sensitivity of flight performance. Thus, we examined 1) activity and thermal sensitivity of anaerobic (lactate dehydrogenase) and aerobic (citrate synthase) enzymes, 2) accumulation of glycogen and lactate, 3) extracellular activity in L. pulchella dragonflies of various ages. LDI activity in nymphs was high (50-60 uM product/minute), decreased by about half in teneral, and in mature was almost nonexistent. Citrate synthase activity increased from very low in nymphs to levels in excess of 60 uM product/minute in mature. However, glycogen content and lactate accumulation were consistently low for all developmental stages, and the rate of fatigue was indistinguishable between nymphal and mature insects. These results indicate that even though tenevals possess an unusually high level of LDH activity for an adult insect, they do not appear to utilize the full anaerobic pathway. Ontogenetic changes in energy metabolism do not appear to cause the shift in thermal sensitivity of adult flight performance. Supported by NSERC grant B-93-17969.


The effects of the arthropod molt cycle on pedestrian locomotion have been previously unknown. We measured the energetic cost of locomotion, stride frequency, and leg muscle power output at 0.31 km/h in last instar and adult cockroaches (Blaberus discoidalis). The energetic cost of locomotion in adults began high at 6.30 ml O2/gm, and declined after the first two weeks of adulthood to an adult value of 4.33 ± 0.21 U/gm. The energetic cost of locomotion for juveniles did not change during the first three weeks following the molt, and was indistinguishable from adult values on days 14 and 21. This result indicates that juvenile and adult values were not obtained by the same strain of cockroaches, and that the rate of fatigue was indistinguishable between juvenile and adult insects. These results indicate that even though tenevals possess an unusually high level of LDH activity for an adult insect, they do not appear to utilize the full anaerobic pathway. Ontogenetic changes in energy metabolism do not appear to cause the shift in thermal sensitivity of adult flight performance. Supported by NSERC grant B-93-17969.

39.13 ENVIRONMENTAL AND PHYSICAL DETERMINANTS OF MUSCLE PERFORMANCE CAPACITIES TUESDAY.
40.1 ONTOGENY OF CARDIAC FUNCTION IN CRUSTACEANS: John L. Spicer & David Marritt, Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2QJ, U.K.

The ontogeny of cardiac function has been studied in three species of crustacean arthropods. In the brachyuran *Artemia franciscana* heart formation and consequently the ontogeny of cardiac function was postembryonic. Instant heart rate (fH) increased with increased development, and was insensitive to changes in environmental temperature (24 to 32°C). When differentiation of cardiac tissue remained complete an inverse relationship between fH and development was noted, and heart rate was then found to be sensitive to temperature change. Individuals of different developmental stages were exposed to waterborne acetylcholine (ACH: $10^{-5}$ to $10^{-3}$ M), but there was no effect on fH, in both the cladoceran *Daphnia magna* and the amphipod *Gammarus duebeni* although cardiac function was present before hatching, the same pattern of fH changes was observed: an initial steady increase followed by a slow decrease with development. The fH of early embryonic amphipods was not affected by exposure to waterborne ACH although fH of pre-hatch individuals was affected. Interestingly fH of *Daphnia* was not affected by exposure to waterborne ACH until sometime after hatching. It would appear that despite the different cardiac structures found in each of the three crustacean groups examined, there were similarities in the ontogeny of cardiac function. Furthermore, there is some evidence that at the onset of cardiac activity fH may be independent of environmental influences.

40.2 HEART RATE DURING DEVELOPMENT IN A REPTILE: EFFECT OF TEMPERATURE. U. P. Birchard and C. L. Reiber, George Mason Univ., Fairfax, VA 22030 and Univ. of Nevada, Las Vegas, NV 89154

Reptiles represent the only vertebrate group in which heart rate (fH) during development remains undescribed. This study examined fH and body mass over the course of development in snapping turtles (*Chelydra serpentina*) at 24 and 29°C. Heart rate early in development (-25 days) was significantly higher in eggs incubated at 29°C. After day 25 fH in 29°C eggs dropped steadily until hatching. Heart rate in 24°C eggs remained relatively steady during most of incubation and then declined significantly at both temperatures as hatching approached. The decrease in fH is not as drastic as hatching approaches suggests a decline in oxygen transport. This would be consistent with hypoxia, as in other vertebrates, being an important stimulus for hatching in reptiles.

40.3 BRADYCARDIA DURING EMBRYONIC DEVELOPMENT IN DOMESTIC FOWL WITH C TO CUS MUTATIONS. Warren Burggren, Robert Howe and Stephen Warburton, University of Nevada, Las Vegas, NV 89154

A highly predictable late embryonic bradycardia (relative to normal White Leghorn chickens) has been documented in chicken strains with C locus mutations. The basis of the bradycardia remains unknown, but it clearly is related to a mutation at the C locus, which contains the structural gene for tyrosinase. When compared to the heart rate of normal White Leghorn chickens, the heart rate of the C locus mutants decreased by 10-12% at day 18 of incubation. fH at C locus mutants showed a 10-12% reduction in heart rate during the last 4 days of incubation. Embryonic mortality occurred in both mutant and normal strains; a significant bradycardia (when compared with surviving embryos of the same strain) developed on the day before death in mutant but not White Leghorn strains. The bradycardia does not affect embryonic oxygen consumption (about 0.2 ml egg$^{-1}$ ad day 14, and 0.4 ml egg$^{-1}$ ad day 20), which shows only minor differences between strains that can be attributed to differences in embryonic mass on days 18-20. This chicken strain may be a useful animal model for determining how heart rate abnormalities affect cardiovascular function in late fetal development.

40.4 CONFOCAL FLUORESCENCE EXAMINATION OF AVIAN LUNG LYMPHATIC DRAINAGE. John A. Malinowski*, Christopher T. Lancaster* and W. Jeffrey Westphal, Section of NPB, UCD, Davis, CA 95616

Little information exists with regard to the control of lung fluid balance in birds. The response of the lungs of Gallus to acute extravascular fluid volume expansion is similar to that in mammals with gas exchanging regions of the air-blissed barrier protected against fluid accumulation caused by increased microvascular filtration (Widder et al. Resp Physiol 91:125 (1993)). In this study we employed confocal microscopy to elucidate the normal pathway of lymphatic drainage in the lungs of Gallus. White Leghorn cocks (2-4 kg body weight) were anaesthetized with sodium pentobarbital (30 mg/kg, i.v.) and allowed to breathe spontaneously through the cannulated trachea. After a brief baseline period, 2 ml of 5% FITC-Dextran 20 (20,000 mol wt) was given i.v. as a "permeant" tracer and allowed to equilibrate for 60 min. At the end of this period 2 ml of 5% RITC-Dextran 70 (70,000 mol wt) was given i.v as an "intravascular" tracer. After 10 min the animals were killed by anesthetic overdose and the lungs were immediately fixed by tracheal instillation at 25 cm H2O of 4% paraformaldehyde in buffered avian Ringers. Confocal examination of the distribution of FITC- and RITC-Dextrans within the lung suggests that drainage of the interstitium of the tertiary bronchial capillary zone may constitute an important component of liquid and solute clearance from this gas exchanging region of the avian lung.

The heart rate of developing avian embryos within the egg shell is determined by measuring electrocardiogram, impedance cardiogram, blood acoustocardiogram (BAG), and arterial blood flow velocity (ABF). While the former two are electrical signals measured invasively by inserting needle electrodes into the egg through the shell, the heart rate can be determined even in an early period of incubation. Both BAG and ABF are acoustocardiographic signals detected from the outside of the shell, offering noninvasive measurement for arrhythmia and heart rate during late period of incubation. Although a condenser microphone has to be installed air-tightly on the shell, we found that BAG signal was less contaminated with somatic movements of embryos than that of ABF. In addition, it was found that the installation of microphone on the shell exerted no adverse effects on heart rate determination in chicken eggs. Taking advantage of ACG measurement, we constructed a noninvasive, long-range measuring system of instantaneous heart rate of developing embryos and measured the heart rate every heartbeat during prolonged period every day from day 12 of incubation. Variability of instantaneous heart rate increased with embryonic development, and characteristic bradycardia was observed in the late chick embryos which may be related to development of vagal function.


Incubation in many species of birds is characterized by pre-incubation egg storage and turning of eggs during incubation. The present study was designed to elucidate the effects of prolonged pre-incubation egg storage and lack of turning on embryonic development with reference to oxygen consumption, heart rate, oxygen pulse, embryonic mass (wet and dry) and water content. Two series of experiments were carried out with chicken eggs. (1) Developmental patterns of oxygen consumption, heart rate and oxygen pulse were determined noninvasively in individual embryos during the last half of incubation. The developmental patterns of these variables were significantly changed in both protractedly stored eggs and those stored for various periods before incubation. In addition to oxygen consumption, the developmental patterns of wet mass, dry mass and water content of embryos and the relationships between these variables indicate that prolonged storage not only impedes embryonic development but also affects adversely physiological functions of embryos.


To further examine the relationship between coronary perfusion and cardiac performance, cardiac output (Q), coronary blood flow (Qcor), and dorsal aortic blood pressure (Pda) were measured in resting and swimming (0.5 - 1.0 bl s⁻¹) rainbow trout (Oncorhynchus mykiss) during normoxia and hypoxia (PO2 approx. 10 kPa). In normoxic trout, changes in cardiac performance variables were observed as the swimming speed was incrementally increased. At 1.0 bl s⁻¹ Qcor and cardiac power output had both increased by approximately 110%, and coronary artery resistance (Rcor) had decreased by 35%. During hypoxia, resting Rcor was 30% higher and Qcor was 20% lower, as compared with normoxic values. In hypoxic swimming trout, the maximal levels of Qcor (155% increase) and Rcor (50% decrease) were recorded at 0.75 bl s⁻¹. In contrast, cardiac power output and Q increased by an additional 45% and 20%, respectively, as swimming speed was increased from 0.75 bl s⁻¹ to 1.0 bl s⁻¹. The results indicate that: 1) increases in Qcor parallel changes in cardiac power output; 2) during hypoxia there are compensatory increases in cardiac performance and coronary perfusion; and 3) the scope for increasing Qcor in swimming trout is approximately 150%. In addition, the cholinergic and ß-adrenergic control of coronary perfusion was investigated by injecting atropine and propranolol into swimming trout. Atropine injection into resting trout caused a 20% decrease in Qcor. Propranolol injection into atriope treated trout increased resting Qcor and reduced their ability to increase Qcor and to decrease Qcor when swimming at 1.0 bl s⁻¹.
42.1

DISCONTINUOUS VENTILATION IN DROSOPHILA MELANOGASTER. Adrienne E. Williams* and Timothy J. Bradley. Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697.

Many insects and other arthropods are capable of closing the spiracles of the tracheal system and preventing gas exchange for short periods of time. This behavior, which is termed discontinuous ventilation (DV), can be identified by the periodic release of CO2. In the early literature, DV was thought to reduce water loss, but more recent investigations have failed to find a strong correlation between the presence of DV and insects adapted to dry environments. Using a Sable respirometer system, we have measured CO2 release from individual flies into a stream of dry, CO2-free air. We found clear examples of DV, the most interesting phenomenon observed in this study, in insects adapted to dry environments. We have been able to identify specific species that exhibit DV and have determined the conditions under which DV occurs. Future studies will focus on elucidating the physiological and ecological factors that contribute to DV in these insects.

42.2

FACTORS CONTRIBUTING TO THE UNUSUAL CARBON DIOXIDE TRANSPORT PROPERTIES OF BLOOD IN LAMPREYS. B.L. Tufts and B.A. Cameron*. Dept. of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Unlike most vertebrates, carbon dioxide (CO2) transport in the blood of lampreys is largely dependent on CO2 carriage within the red blood cell. This has led to the use of different methodologies to determine the factors contributing to this unusual strategy for CO2 transport in lampreys. Inactivation of sodium/proton exchange significantly increased the amount of bicarbonate carried within the plasma and reduced that carried within the red blood cells. However, the mechanism for the increase in bicarbonate concentration in the red blood cell membrane remains unclear. Additional studies are needed to better understand the factors contributing to this unusual strategy for CO2 transport in lampreys.

*Supported by NSERC Grant to BLT.
42.3 BLOOD RESPIRATORY PROPERTIES OF SOME AIR BREATHING GOLIES. Nancy M. Aguilar and Jeffrey H. Graham, Scripps Institution of Oceanography, La Jolla, CA, 92037-0204.

It is generally held that the evolutionary transition from aquatic to aerial respiration should be accompanied by shifts in blood respiratory properties including increased $P_{50}$, increased $bohr$ shift, and a decreased oxygen capacity. Although, inter familial comparisons do not show significant differences, various aquatic and aerial breathers in the fish families Osteoglossidae and Erythrinidae. We hypothesized that blood characteristics might also differ among species of the family Gobiidae with differential access to air. In this study, oxygen consumption (OCDs) were determined using biochemistry, foraminifera, Porichthys flaviviridis, and Gillichthys mirabilis, two facultative air-breathe. OCDs were compared between 20 and 35°C, and pH 7.6 and 7.8. Oxygen capacity and blood pH were determined at 25°C. The OCD of E. barbarus is right shifted at both pH 7.6 and 7.8. $E$. barbarus has a higher hematocrit and higher hemoglobin concentration than $G$. mirabilis. Expansion of the approach to include other gobies with various air-breathing specializations will further define the extent of the evolutionary process on blood characteristics on this family.

42.5 OXYGEN CONSUMPTION, NITROGEN EXCRETION, BRAIN MONOAMINES AND BRAIN METABOLITES IN HADROCAL AMPHIBIANS EXPOSED TO COPPER. Gudrun Debeck', Hans De Smet*, Department of Zoology, La Trobe University, Melbourne, Victoria, Australia 3083.

We have previously shown that copper concentrations of 0.34 and 0.84 pM have no significant effect on the swimming performance of fish. At 0.84 pM, oxygen consumption increased significantly immediately after exposure to copper. Also, serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), norepinephrine, epinephrine, and norepinephrine concentrations were affected. After one week of exposure to copper, significantly increased levels of 5-HT and 5-HIAA were found in the brain and blood of fish exposed to copper. In addition, 5-HT and DA could be seen in the group exposed to 0.84 pM, while in hypothalamus only 5-HT was present. It is concluded that even at low concentrations, copper exposure of common carp causes decreased brain 5-HT and DA levels, two neurotransmitters involved in feeding behaviour and locomotor control in fish. Furthermore, $O_2$ consumption is at its peak during the day or induced by hypoxia. (Quebec Lung Association)

42.6 RESPONSE OF REPTILIAN INTRAPULMONARY $CO_2$ RECEPTORS TO $NH_3$ BEFORE AND AFTER ACETAZOLAMIDE ADMINISTRATION. Richard D. Talman, Jr, Texas A&M University, Department of Physiology, The Ohio State University, Columbus, Ohio 43221.

The pine snake (Pinophis melanoleucus) has been shown to have carbon dioxide sensitive primary afferents in its lungs. These intrapulmonary $CO_2$ receptors or IPC discharge in inverse proportion to the arterial $PCO_2$. The intrapulmonary $CO_2$ receptors of birds and fish have been shown to occur both among aquatic and air-breathing animals. We hypothesized that such receptors might exist in the lungs of reptiles, in the pine snake (P. melanoleucus). We observed that the administration of acetazolamide (a diuretic with weak anticholinergic activity) caused a gradual and sustained increase in discharge. In addition, $NH_3$ has been shown to enhance the sensitivity of these receptors. Therefore, we suggest that the respiratory control of $NH_3$ and $CO_2$ is maintained by a gradual and sustained increase in discharge. The purpose of the present study was to determine the response of IPC to $NH_3$ before and after the blockade of carbonic anhydrase with acetazolamide. Pine snakes (avg. wt. 200 gm) were anesthetized (pentobarbital 24 mg/kg S.Q.) and unilaterally ventilated. For drug infusion, a non-oedematous catheter was placed in the pulmonary artery close to the heart. In most, but not all IPC, a bolus injection of 10 mg $NH_3$ resulted in a rapid increase in discharge followed by a decrease or no change lasting up to 3 hr. Eight IPC were studied. Six IPC responded to $NH_3$, while two IPC responded to $CO_2$. The response to $NH_3$ was greater than to $CO_2$. It is concluded that $NH_3$ and $CO_2$ receptors are present in the lungs of the pine snake. (Lake Tahoe Medical Association, California State Water Project Fund, and private donations.)
42.3

POSTURAL-VENTILATORY INTEGRATION IN THE PANTING DOG.

Dennis M. Bramble*, Tavis A. Jenkins, and Joth Feller. Univ. of Utah, Salt Lake City, UT 84112

Cineradiography and electromyography were used to investigate the biomechanics of post-exercise panting in standing dogs. This approach allows detailed correlation of the kinematics of key thoracic structures with airflow. Ventilation and postural interactions of the thoracic complex are highly integrated and the mechanics of breathing differ markedly from those of resting mammals. Respiratory displacements of the diaphragm and rib cage induce synchronized accelerations of the trunk. The latter represents a potentially significant, but previously unrecognized addition to the work of breathing. Above respiratory frequencies of 1.5 Hz, airflow is generated by the gill beat and rib cage acceleration rather than velocity. Kinematic details strongly imply that panting dogs may exploit the kinetic inertia of the visceral mass to help drive the long ventilation. Finally, panting dogs appear to be able to adjust the relative phase relations of their apical and diaphragmatic pulmonary lobes, thereby achieving asynchronous lung ventilation just as in running dogs (Bramble and Jenkins, Science 262:235). The ability to shift the phasing of these lobes (sum synchronously to fully asynchronous potentially involves modulation of the impedance of the anterior chest wall overlying the apical lobes through changes in the recruitment of the muscular suspensory sling of the thorax.

Support: NSF IBN 9118610

42.11

EFFECTS OF CALCIUM DEFICIENT DIET AND ACETAZOLAMIDE ON PORE AND MAMMALIAN KNOK FORMATION IN AVIAN EGG SHELLS. D. E. Imbriale, M. Costello, and S. C. Hempleman. Dept. of Biological Sciences, Univ. of Northern Colorado, Greeley, CO 80639 and Dept. of Medicine, University of California, San Diego, La Jolla, CA 92093.

In a previous study of eggshell gas exchange in Gallus domesticus (Bleibrot and Hempleman, Respir. Physiol. 95:11-20, 1994) calcium deficient diet increased eggshell weight without concomitance (30%) and was accompanied by a 21% decrease in eggshell weight (Lt). In the other hand, acetzolamide increased Aop+ 200% (first day after administration) and post-infusion increase in total functional pore area (Ap) to 7% in non-exposed mammalian knok formation. Scanning electron microscopy was used to study mammalian knok formation under calcium deficient diet and acetzolamide on the channels between the pores and It was also evident. The purpose of this study was to investigate the effects of calcium deficient diet and acetzolamide on mammalian knok formation in the same eggshell. Standard-point-counting procedures were used to estimate the number and pore radius. Pore number 186.27 pores/cm2 (P < 0.01), and several isolations mammalian knok formation. We conclude that calcium deficient diet increases Aop+ by eggshell thinning with little effects on pore and mammalian knok formation. On the other hand, acetzolamide primarily increases Aop+ by increasing pore number with severe effects on mammalian knok formation. (Supported by NIH HL7731).

42.13

ACID-BASE TRANSFERS DURING ACIDOSES IN THE EURYHALINE LONG-HORNED SCULPIN; EFFECT OF EXTERNAL SALINITY.

James B. Clasborne*, S. Bellows, E. Perry. Dept. of Biology, Georgia Southern University, Statesboro, GA 30460 & MDI Biological Laboratory, Saltsbury Cove, ME 04672

Acid-base transfers across the teleost gill (Na+/H+, Na+/HCO3- exchange) may be influenced by the concentration of external counter ions. Sculpin (Myoxocephalus rosenbergii) can compensate for an infused acid load over 12.4 h when in seawater (SW) or dilute (60%) SW, but excretion is impaired in very dilute water (4%). Pre-adaptation to 60% SW allows the animals to recover from the acidosis more quickly. We studied the effect of acid infusions (7 mcg kg⁻¹ HCl) in pre-adapted fish exposed to low external [Na+] or [Cl·] conditions. Sculpin in low Na⁺ water, took up H⁺ at a rate of 0.27 ± 0.04 mcg kg⁻¹ h⁻¹ (µ ± S.E.) during the post-infusion period. When external Na⁺ was restored, ΔH⁺ changed to an excretion of 0.18 ± 0.05, identical to the 20% seawater group. Fish in low Cl⁻ water exhibited a rate of 0.32 ± 0.02. An infusion of similar to 20% seawater animals) and lost -75% of the load in the first 4 h. Thus, external Na⁺ was critical for the net transfer of H⁺ while a reduction in ambient Cl⁻ increased the excretion of the administered acid. Acid excretion was directly related to external Na⁺. We hypothesize that a tranabsorptive Na⁺/H⁺ exchange is operating in opposition to a gill Cl⁻/HCO₃⁻, transfer and that a loss of ambient Cl⁻ incases the excretion of the administered acidosis. Acid (similar to 20% seawater) and severe effects on pore number 186.27 pores/cm² (P < 0.01), and several isolations mammalian knok formation. On the other hand, acetzolamide primarily increases Aop+ by increasing pore number with severe effects on mammalian knok formation. (Supported by NIH HL7731).

42.40

GAS COMPOSITION IN MIDDLE EAR CAVITY VS SUPERIOR VENA CAVA BLOOD OF THE GUITRINA PTO. A. Alia, D. Ilnitzi, H. Nover, D. Lovit, M. Harel*, and J. Samit. Departments of Zoology and Biology, Tel Aviv Univ., Tel Aviv 69978, Israel

Middle ear cavity and superior vena cava (SVC) gas samples were obtained from a poorly ventilated gas pocket with rigid walls and close to atmospheric Pb. This predicts almost identical steady-state values for CO₂ and O₂, and thus very little contribution to the Pb of the SVC gas, which indicates that venous blood leaving it (Pco₂; Pvo2) respectively, and a pronounced PbHCO₃/PbCO₂ difference, with high PbHCO₃. However, whether MB gas exchange can be observed in the SVC, is not established. A calibrated membrane-covered mass spectrometer probe (auction:2ml/s) is located via the bulla into the ME of 18 anesthetized guinea pigs, on the opening sealed. In 4 min a 2nd probe was introduced into the superior vena cava. Gas composition was monitored until steady-state was achieved. Pressures were calculated using PHO, Pb and Pb saturation. The PbHCO₃/PbCO₂ was assumed to equal that of alveolar gas and readings of other gases in blood to be proportional to it. Values (mean±SD; 15±15/0): PHO₂ =42±5mmHg; pco₂=40±4mmHg; Pvo2=30±0mmHg; Pco₂=61±20mmHg; Pbo2=56±8mmHg. Time constants (TC, s):14s. For steady-state establishment were 13±16s and 37±5s for O₂ and O₂ respectively. Thus the data reflect mixed sources of blood but the large PbHCO₃ difference is close to predicted. The PbHCO₃/PHO₂ difference is insignificant, not proving large diffusive gas/blood resistances.

42.12

CHANGES IN NIT RIAS CUE ON PLASMA PROTEINS. M.L. Halpern, S. Vuagniaux*, and R.K. Randol, Renal Division, St. Michael’s Hospital, Toronto, Canada.

Analysis of a clinical sample with metabolic acidosis and a very large anion gap in humans (AG) led to a major conceptual problem-there appeared to be an accumulation of many amino acids, particularly serines and cysteines, and a number of unidentified products.

CASE: A 62 year old woman presented with a 2 day history of polyuria and polydipsia to quench thirst. She drank a large quantity of sweetened beverages. She had a diagnosis of NIDDM, but an oral glucose tolerance insulin secretion was normal. She was drowsy and had a severe degree of ECF volume contraction. Her mean arterial pressure was 127/82 (75 mmHg), and her heart rate was 117 bpm with 41% systolic ejection fraction. Her blood was only 3 mmol/l and the pH was 7.2. The patient received insulin and a new of 4 liters of intravenous saline. To examine the relationship between amines and plasma pH, the sample was collected every 2 hr for 24 hr. Over the 24 hr, the AG decreased by 22 mmol/l. Ammonia was lost in the urine as could be determined from the rate of excretion of ammonia (Na⁺ + K⁺ + NH₄⁺ + Ca²⁺ + Mg²⁺ + Sr²⁺ + Ba²⁺) in the first 30 min was 5 mmol/l. These changes in the AG could not be accounted for by changes in systemic concentration or pH in plasma. Based on HCO₃⁻ and AG values, the changes in the AG seemed to reflect an anion with a volume of distribution that is intravascular. Taken together with our previous studies that demonstrated that the AG is increased by a low intravascular volume, we speculate that these changes in the AG might be due to a compound, other than or in addition to albumin, with a net anionic charge. Changes in its concentration or its volume of distribution may serve to drive intravascular volume via the Starling effect during marked ECF contraction.

42.14

LACTATE TRANSPORT ACROSS WHITE MUSCLE CELL MEMBRANES OF RAINBOW TROUT IN VITRO. Yuxian Wang, Christina F. Mlsnak*, George A. Heithagen, and Chris M. Wood, Dept. of Biology and Medicine, McMaster Univ. Hamilton, Ont, Canada, L8S 4K1.

An isolated perfused tail-trunk preparation was used to examine the release of lactate from fast-exercised white muscle. The transmembrane pH gradient was manipulated by variable perfusate pH (approx. 5.4, 7.0, and 7.4) via adjusting HCO₃⁻ while maintaining Pco₂, and the electrical gradient (Eh) was changed by increasing perfusate K⁺ from 2mm to 15mm. Transmembrane lactate distribution is neither pH nor Eh dependent. This suggests that the membrane is highly differentiated and is responsible for Lac- and carrier-mediated Lac transport could be involved. Based on this finding, specific blockers: a-cyano-4-hydroxynicinnamic acid (CIN), 4-Acetamido-4'-isothiocyanatoaniline 2,2'-disulfonic (STIS) and amiloride were used to identify the potential role of various ion transporters in lactate uptake in CIN, but no blocker of both Lac⁻/H⁺ cotransporter and Lac/H⁺/Cl⁻ cotransporter, respectively significantly reduced Lac efflux from post-exercised muscle while STIS, a more specific blocker for Lac⁻/H⁺/Cl⁻ exchange, did not show any significant effect on Lac efflux. This suggests that Lac⁻/H⁺ cotransport is involved in Lac efflux. The possible roles of these transporters in the re-uptake of Lac from extracellular fluid into the white muscle are currently being investigated (Supported by NSERC).
42.15

ERODIENIC CONSEQUENCES OF INTRACELLULAR ACIDITY IN
SUSTAINED RABBIT PROTRACTOR MUSCLE. C.A. Combo and M.A.
Ellington. Dept. of Biological Science. Florida St. Univ.,
Tallahassee, FL 32306-3050.

We have evaluated the impact of experimental reductions of
intracellular pH and its effects on the performance of the
renal protractor muscle (rpm) of the marine gastropod,
Bathyomphalus, using phosphorus NMR spectroscopic
approaches. Muscle bundles were placed in a homebuilt
probe and fully-relaxed NMR spectra were acquired at 10.9
MHz. It was possible to "clamp" pHi in various acidotic
states by superfusing the muscle with 5, 10 and 15 mM
medium 5.5 dimethyl-amine-3, 4-dione (DMO) in buffered
artificial seawater (BASW). Superfusion with DMO
resulted in consistent reductions of pHi (7.5-7.6, 6.8
and 6.6, respectively) which persisted for at least 4h. During
the acidotic transitions, [ATP]/[ADP], [Pi] and pHi increased
incrementally with reduced pHi. All of the above effects
but also produces a disequilibrium of the ATPase/synthase
reaction. Supported by NSF grant (IBN-9104548).

42.17

INTRACELLULAR MUSCLE pH RECOVERS RAPIDLY IN
GHOST CRABS FOLLOWING EXERCISE TO EXHAUSTION.
R.B. Weinsteín1, J.F. Harrison2 and R.J. Ful1. 1 U.C. Berkeley,
Berkeley, CA 94720, and 2Arizona State Univ., Tempe, AZ
85287.

Exercise to exhaustion results in metabolic disturbances that
inhibit subsequent exercise. We examined the rate of recovery of
intracellular muscle pH in the ghost crab, Ocypode quadrata
(mean weight = 28.6 g), following exercise to exhaustion (2.6
min) on a treadmill at a speed of 0.3 m/sec and a body
temperature of 24°C. At the time of fatigue, leg muscle lactate
concentration was 5-fold above resting levels. Venous
hemolymph pH decreased from 7.65 at rest to 7.17 at fatigue and
pCO2 increased from 12.0 to 25.4 torr. Intracellular muscle pH
was maintained above resting levels (3.0-fold) and intracellular
muscle pH returned to resting levels within 30
min of recovery. Muscle lactate returned to resting levels
within 5 min of recovery. Muscle lactate concentration was
3-fold above resting levels) following 30 min of recovery. The rapid
recovery of intracellular pH measured for the ghost crab is 2 to 4
times faster than values reported for other crustaceans. We
propose that rapid recovery from metabolic disturbance associated with high intensity activity contributes to the
ghost crab's capacity to increase its performance limits by moving intermittently (i.e., alternating brief movements with brief pauses).

PHYSIOLOGICAL ECOLOGY

43.1

GONADAL STATUS AND THE ACQUISITION OF THE "WINTER"
PHENOTYPE IN THE H. R. NAVY, B. A. Gerber, and Milton H. Stetson. University of Delaware, Newark, DE.

This study was designed to examine the effects of gonadal size on the
acquisition of the "winter" phenotype in adult male collared lemmings
(Dicrostonyx groenlandicus). Lemmings were born and raised to
weaning (19d), all lemmings were placed in LD for 10 weeks. At 10 weeks
postweaning, lemmings were transferred to SD. Body mass and pelage color
were rated biweekly. The experiment was terminated on week 20 and data
were collected. Proestrus was determined on week 20. Proestrus
appeared to affect P (0.30) the short photoperiod-induced growth of adult lemmings. Pelage color was significantly
(P < 0.05) affected by proestrus photoperiod; lemmings from the Pre SD
group were whiter at weeks 18 and 20. Body color was not significantly
influenced by proestrus photoperiod. At week 10, lemmings from the Pre SD
group had significantly larger testes and seminal vesicles (P < 0.001) than
lemmings from Pre LD. At week 20 (after 10 weeks of SD exposure) lemmings from the Pre SD group showed testicular regression when compared to their
10 week counterparts (P < 0.05) and testes mass was not significantly different
from the Pre LD group (P > 0.14). These results suggest that testicular
regression is conducive to the acquisition of the "winter" phenotype. Thus, collared
lemmings appear to have "uncoupled" the seasonal regulation of somatic changes and reproductive function. (Supported by NSF DCB187-1460)

43.2

ANNUAL CYCLE OF PLASMA LIPIDS IN CAPTIVE STRIPED BASS,
MORONE SARTIUS. Eric D. Lund1, Allen R. Place2 and Craig S. Sullivan2. 1Center of Marine Biotechnology, Baltimore, MD. 2North Carolina State University, Raleigh, NC 27605-7601.

Protein, lipid and fatty acid concentrations in plasma of eight male and
eight female one year old captive striped bass (Morone saxatilis) were
monitored monthly over the course of two reproductive cycles as part of an
effort to investigate the time course of lipid class mobilization and
subsequent deposition in gonads. Total protein levels (44.2 ± 2.67 SE mg/ml,
Range 20.8-66.4) showed seasonal fluctuation, but did not vary with sex.
Total lipid concentrations in the plasma of both males (17.7 ± 0.61 mg/ml)
and females (14.1 ± 0.64 mg/ml) showed seasonal fluctuations with the
lowest levels in late Spring during spawning. Plasma lipids in females were
significantly (p< 0.05) lower than those of the males except during early
ovarian secondary growths. Analysis of the lipid class composition revealed
that the decrease in plasma lipid concentrations in females prior to
spawning is primarily due to a decrease of up to 50% in the phospholipid
component of plasma relative to males. Striped bass vitellogenin was found
to contain approximately 20% lipid by weight with nearly 80% of the lipid
being phosphatidyl choline (PC). Vitellogenin levels previously measured
in these individuals were highest during the winter months and decreased
in the 2 months prior to spawning. Although mature striped bass ovaries are
rich in eicosanoids, no eicosanoids were found in the plasma.

Preliminary analysis of the fatty acyl composition of separated lipid
classes suggests that PC is the primary carrier of the essential fatty acids
(22:6 (n-3) and 20:5 (n-3)) to the gonads.
TUESDAY

43.3

AN EXPERIMENTAL AND COMPARATIVE STUDY OF DIETARY MODULATION OF INTESTINAL ENZYMES IN EUROPEAN STARLINGS (Sturnus vulgaris). Carlos Martinez del Rio, Dept. of Ecology and Evolutionary Biology, Princeton University, Princeton, N. J. 08544 1003

The ability to up-regulate high levels of intestinal maltase activity (the main enzyme involved in the digestion of complex carbohydrates) and the ability to modulate disaccharidases (maltase, sucrase, and isomaltase) and one protease (aminopeptidase-N) in response to changes in nutrient intake. This was studied on diet-fed starlings. The diet containing 52.5% corn starch, a carbohydrate-free diet, and an insect diet. Diet had a significant effect on intestinal maltase activity in starlings. Maltase activity was increased in response to increased protein and carbohydrate intake, respectively. The increase in maltase activity was relatively low with a maximal increase of 30-50% observed in the small intestine.

43.4


Arguments of economical design have suggested a match between nutrient load and uptake capacity in animals. Also, it has been argued that because foods contain toxins, there would be selection against reliance on passive absorption in favor of active absorption resulting from specific transport proteins in the intestinal brush border. We tested the hypothesis that most glucose absorption across the small intestine's brush border is normally by a mediated pathway (i.e., the Na+/glucose cotransporter), and that mediated glucose uptake is matched with dietary loads, in three bird species -- a carnivore - Thylotis haematopus, a granivore - Passer domesticus, and an insectivore/granivore - Luscinia cinerea. Glucose absorption was measured in vivo using the everted-sleeve technique, and passive absorption of L-glucose in vivo using a method adapted from pharmacokinetics. None of the species increased mediated glucose uptake on a higher carbohydrate diet. Estimates of mediated uptake of D-glucose across the brush border membrane in vitro using the everted-sleeve technique, and passive absorption of L-glucose in vivo using a method adapted from pharmacokinetics. Results suggest that passive absorption is substantial. The passive pathway appears to provide birds with a way to regulate their intake and metabolism by adjusting the passive absorption rate of glucose.

TUESDAY

43.7

TEMPERATURE AND THYROID HORMONE LEVELS DURING INCUBATION INFLUENCE METABOLIC RATE AND THERMAL CHOICE OF JUVENILE SNAPING TURTLES. S. O'Steen, Dept. of Biology, University of Chicago, IL 60637

Temperature can influence energy expenditure and thermal preference of adult reptiles. Hypothesised that egg incubation temperature would influence energy use and metabolism of juvenile turtles, and that this influence might be mediated by thyroid hormones. Eggs of the snapping turtle Chelydra serpentina were incubated at 25.1°C, 24.3, 27.2, or 30.5°C. Metabolic rate, measured as oxygen consumption at 25°C three days post-hatching, was significantly higher in animals from the 25°C group compared to those from the 24.3, 27.2, and 30.5°C groups. These data demonstrate that the high water turnover of Alaskan sled dogs mandates a similarly high potential renal solute load. We measured water turnover and factors influencing urine volume and composition in groups of highly trained Alaskan sled dogs. Two groups of 6 dogs (OG) and 6 dogs (BG) were housed in unheated kennels. Body water turnover was estimated using deuterium oxide. Urine and blood samples were collected before, at the midpoint, and immediately after the race. Average ambient temperature was 27°C. The length of the race was 490 km, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog. The high potential renal solute load was 22.5 ± 1.5 kg, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog.

43.8

MATERIAL METABOLISM OF ALASKAN SLED DOGS IN FAVOR OF INCREASED METABOLIC ACTIVITY. Gregory A. Hebel, John R. Burr, and Richard H. Downum. College of Veterinary Medicine, The Ohio State University, Columbus, OH and * Research and Development, The Iams Company, Columbus, OH.

Body water turnover is a measure of body water turnover and factors influencing urine volume and composition in groups of highly trained Alaskan sled dogs. Two groups of 6 dogs (OG) and 6 dogs (BG) were housed in unheated kennels. Body water turnover was estimated using deuterium oxide. Urine and blood samples were collected before, at the midpoint, and immediately after the race. Average ambient temperature was 27°C. The length of the race was 490 km, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog. The high potential renal solute load was 22.5 ± 1.5 kg, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog. The high potential renal solute load was 22.5 ± 1.5 kg, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog.

43.9


Active metal uptake processes at fish gills are temperature dependent, because fish metabolic rate changes as temperature is increased or decreased (Q10 = 3-4). Passive uptake (e.g. diffusion through the gills) is essentially temperature independent over temperatures tolerated by trout, because diffusive flux is dependent on ambient temperature. Arguing for an increase in passive uptake (e.g. diffusion) with increasing temperature, because low temperature tolerance. These data demonstrate that the high water turnover of Alaskan sled dogs mandates a similarly high potential renal solute load. We measured water turnover and factors influencing urine volume and composition in groups of highly trained Alaskan sled dogs. Two groups of 6 dogs (OG) and 6 dogs (BG) were housed in unheated kennels. Body water turnover was estimated using deuterium oxide. Urine and blood samples were collected before, at the midpoint, and immediately after the race. Average ambient temperature was 27°C. The length of the race was 490 km, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog. The high potential renal solute load was 22.5 ± 1.5 kg, with total body water of 0.71 ± 0.02 kg/kg/dog and 0.68 ± 0.05 kg/kg/dog.
43.9

EVAPORATIVE WATER LOSS IN NINE INSULAR LIZARD POPULATIONS OF THE GENUS ANOLE CRISTATELLUS IN THE BRITISH VIRGIN ISLANDS. Razi Dm'tel, Gad Perry* and James Lefkoe. Zoology Dept., Tel Aviv University, Tel Aviv 69978, Israel; Zoology Dept., Univ. of Texas, Austin, TX 78712; The Conservation Agency, 6 Swinburne St, Jamestown, RI 02835.

We studied evaporative water loss (EWL) and integumentary resistance to water loss (Rsw) in eight insular populations of the lizard Anolis cristatellus and in one population of Anolis erneestwilliamsi in the British Virgin Islands. There was a strong negative correlation between habitat aridity and EWL (ranging from 10.3 to 1.5 mg g⁻¹ h⁻¹), and a positive correlation between habitat aridity and Rs (29-199 g cm⁻²). EWL and Rs of A. ernestwilliamsi were similar to what would be predicted for a similar sized A. cristatellus living in the same habitat. The Guana Island population of A. cristatellus was significantly different from all other populations. Most of the observed variability may be attributed to phenotypic plasticity, but genetic differentiation may be responsible for the distinction of lizards from Guana.

43.10

AEROBIC CAPACITY OF RED JUNGLE FOWL: ONTOGENY, REPEATABILITy, AND EFFECTS OF PARASITES. Mark A. Chappell, Marlene Zuk, and Tor Johnsen. Biology Department, University of California, Riverside, CA 92521

Aerobic capacity (maximum O₂ consumption; VO₂max) is the basis of power production in endotherms and hence is a good index of overall metabolic performance. Little is known about individual consistency of VO₂max (especially during ontogeny), or the effects of routine infections on VO₂max. We examined VO₂max (elicited by exercise in a running wheel) in the red jungle fowl Gallus gallus. One to three weeks after hatching, half of a cohort of 90 chicks were infected with the nematode Ascaridia galli, a common intestinal parasite of galliform birds. A. galli infection significantly depressed VO₂max and body mass in 9-day old chicks but had no measurable effect in adults. Males had significantly higher VO₂max than females in both adults and chicks. The VO₂max of adults was highly repeatable (r=0.5 - 0.91; P <0.05) over intervals from 2 h to >60 days. However, performance rankings of chicks (after correction for body mass) were not repeatable after growth to adulthood.

43.11

HERITABILITY OF SPEED, ENDURANCE, AND MAXIMAL AND BASEAL RATES OF OXYGEN CONSUMPTION IN HOUSE MICE. Robert Hill, Plymouth, PL1 2PB, UK

We investigated the quantitative genetic basis of maximal locomotor performance and activity metabolism using a genetically directed analysis. We used a combined parent-offspring, half-sib, full-sib breeding design, with crossfostering, to estimate narrow-sense heritabilities (h² = additive genetic variance/covariance phenotype variance) and genetic correlations (r) to genetic analyses by maximum likelihood, we used multiple regression to remove the effects of measurement block, sex, age at testing, and other relevant covariates. Residual log swimming endurance showed high heritability (h² = .30, chi² = 6.07, df = 1, P < 0.05) and did not differ significantly from the mean. Phenotypic correlations between speed and endurance were low (r = 0.17, P = 0.80), and the additive genetic correlation was large, negative, and statistically significant, suggesting a necessary trade-off. Both residual maximal oxygen consumption (VO₂max) and basal metabolic rate (BMR) showed low heritability. VO₂max and BMR were uncorrelated phenotypically (r = 0.058, P = 0.99), but r² was strongly positive and marginally significant. This apparent genetic coupling is consistent with the "aerobic capacity" model for the evolution of endothermy. Supported by NSF grants IDN-9111605 and IDN-9157260 to TG.

43.12

IS LOCOMOTION MORE COSTLY IN THE COLD RELATIVE TO INACTIVITY? Eileen Zielke, All Diaz* and Matthew Leduc*. Colgate University Biology Department, Hamilton, NY 13346

Endothermic animals active at cold ambient temperatures must allocate energy to meet both trophotrophic demands and energy required for locomotor activities. The purpose of this study was to investigate the contribution of exercise-generated heat to thermoregulation by American Goldfinches and Eastern House Finches during cold stress. We tested the hypothesis that during cold exposure, the metabolic heat production of exercising birds will not differ significantly from the metabolic heat generated by resting birds exposed to similar convective conditions. To test our hypothesis, energy metabolism and body temperatures of sedentary and active birds (running on a treadmill) was measured at a range of air temperatures (-10 to 35°C) and under still air and moderate wind. Energy metabolism was measured as the rate of oxygen consumption in an open flow resorometry system. The average specific metabolic rate for resting birds was 154.2 ± 20.6 ml of O₂/hr and that of exercising birds was 157.3 ± 11.5 ml of O₂/hr. These results support our hypothesis and the concept energy is conserved by exercising birds in the cold. Exercising birds do not incur more of an energetic cost associated with activity at low temperatures in comparison to an inactive bird exposed to similar convective conditions. We conclude that the complementation of exercise-generated heat to thermoregulation may provide a means by which animals can minimize energy expenditures during cold stress and locomotor activities such as foraging. Supported by Colgate Research Council Grant and NSF Research Planning Grant No. IIB-930571.

43.13

IS BARNACLE EGG HATCHING PHEROMONE AN EXCRETORY METABOLITE? Anthony S. Clare, Marine Biological Association, Citadel Hill, Plymouth, PL1 2PB, UK

The eggs of the borearctic barnacle Semibalanus balanoides are brooded in the mantle cavity of the adult. The eggs hatch and are liberated in synchrony with the spring phytoplankton bloom, and in response to the release, by the adult, of the egg hatching pheromone (EHP). 10,11,12-trihydroxy-5,8,11,14,17-eicosatetraenoic acid. Since the putative precursor of EHP is eicosapentaenoic acid (EPA), and this polyunsaturated fatty acid is common in marine lipids, and thus the diet of barnacles, it is feasible that EHP is a dietary metabolite. However, the following findings suggest that this hypothesis should be rejected. First, barnacles fed on a diet of the diatom Skeletonema costatum did not excrete radiolabelled EHP. Based on these results, it now seems likely that EHP is metabolised to EHP by this class of enzyme. Current efforts are directed at determining the nature of the LPO and examining the expression of LPO mRNA in barnacle tissues.

43.14

SNAPPING TURTLE EGGS THAT GAINED MASS DURING INCUBATION HAVE MORE ALANTOIC FLUID THAN EGGS THAT DID NOT CHANGE IN MASS. Thomas A. Davis, Dept. of Biology, Loras College, Dubuque, IA 52004-0178

Previous studies have shown that snapping turtle eggs incubated in wet sand or vermiculite at 28°C usually absorb water during incubation. Though others have shown that water content of yolk was higher than water content of albumen when egg mass was not affected when egg mass increased, this volume of yolk water did not account for the total egg mass gained. To determine destination(s) of absorbed water, snapping turtle eggs were incubated at 24°C in wet (0.65±0.07 g water/g dry sand), but not different between groups.Embryos from WSE had significantly larger yolk water content and allantoic fluid volume than DSE embryos. Total osmolarity and [Cl⁻] of allantoic fluid were lower in WSE which supports the hypothesis that allantoic fluid is another site of deposit of absorbed water throughout incubation. Physiological implications of elevated water content of allantoic fluid and yolk on embryonic development await further investigation. This research was supported by a grant from the Iowa Academy of Sciences.
College.
on hatchling mass.

PERWRMANCEJN FISH: AND4TEiXATED STUDY OF M~LFCUL+AR AND CELLULAR

HATCHLING TURTLE GROWTH IS INFLUENCED BY EGG INCUBATION

CONDITIONS. Kirk Miller. Franklin & Marshall College, Lancaster, PA 17604

Snapping turtle (Chelydra serpentina) eggs were incubated on substrates with water potentials of -150 or -800 kPa and at temperatures of 26 or 29 °C. Eggs from cool and wet treatments produced heavier hatchlings compared with eggs from warm and dry treatments. Hatching mass was also influenced by initial egg mass and clutch of origin.

Turtles grew to different sizes. The most important predictors of hatchling mass were the temperature at which eggs were incubated, the temperature at which growth occurred, and the clutch of origin. After 8 months of growth, turtles from eggs incubated at 26 °C were heavier than those incubated at 29 °C, independent of the influence of incubation temperature on hatching mass.

Supported by the Committee on Grants, Franklin & Marshall College.

MUSCLE AND LOCOMOTOR ADAPTATION

EVOLUTIONARY RESPONSE OF SWIMMING PERFORMANCE IN

GUPPIES TO DIFFERING PREDATION INTENSITIES. Alastair J. Cullum. U.C. Irvine, Irvine, CA 92717

Locomotor ability has often been perceived as important in the outcome of such types of predation experiments. However, few studies have actually examined selection on locomotion within generations, and fewer if any have looked for genetic responses to predation pressure. Natural populations of guppies (Poecilia reticulata) in Trinidad provide a natural experimental system to examine the evolutionary effects of high vs. low predation intensities on two forms of escape swimming. The first is the rapid (3-4 s) start, a rapid-response "kick" escape maneuver that involves both the horizontal middle躯干 and anterior trunk segments. The second is the subsequent burst (or sprint) swimming used by the fish to move greater distances. Lab-reared fish from replicate populations of high and low predation intensities were filmed using high-speed video while escape responses were elicited via a standardized stimulus.

Video frames were then digitized to yield data on velocities and distances moved during different phases of the escape response. Work to date suggests that rapid (3-4 s) start is not always present in the different populations. The second is the subsequent burst (or sprint) swimming is higher in the line with high predation performance. These findings were corroborated by changes in organismal kinematics during swimming. For example, in goldfish only, fast muscle twitch contraction kinetics were affected by acclimation temperature in goldfish (P<0.0001), whereas only angular velocity was significantly correlated with the selection for high response in a wedge-shaped curve similar to that of rabbit skeletal muscle. Fish cardiac RYR displays a similar activation threshold but shows optimal [3H]ryanodine binding at 100 μM Ca2+ and inactivation at 3 mM Ca2+. In contrast, the functional implications of this Ca2+-independent activation of channel activity in the α and β isoforms from fish. In conclusion, we identified a similar pattern in planar lipid bilayers, we identified two different calcium channels based on conductance properties and calcium dependence of calcium entry. The possible role of the physiological differences between RYR isoforms are discussed.
44.5 HIGH FAT DIET IMPROVES AEROBIC PERFORMANCE BY BUILDING MITOCHONDRIA. C. Richard Taylor, Hans Hegl, C. Kennedy, T. Valensuki, T. J. Roberts and P. Weyand.
CSF, Harvard University, Old Causeway Rd, Bedford, MA 01730 and Dept. of Anatomy, Univ. Berne, Berne, Switzerland.

Eating a high fat diet increases rates of fat oxidation in exercising humans, pigs and rats. We have found that during exercise most of the fat is supplied to the muscles cells. Every fat droplet is in direct contact with a mitochondrion, suggesting that the fatty acids are released directly from the droplet into the mitochondria for oxidation, circumventing transport problems associated with low solubility. We hypothesized that the area of contact would increase in direct proportion to fat oxidation when animals were fed a high fat diet.

To test this hypothesis we measured maximal aerobic capacity and maximal rate of oxidation while animals were fed a normal commercial diet (25% of the calories from fat). Then we fed dogs a high fat diet containing 45% of the calories from fat while holding their training constant. Maximal rate of fat oxidation increased dramatically on this diet, reaching 3343 μmol O₂/min/kg (±332 SE) after 4 to 6 weeks—an increase of more than 50%. Maximal aerobic capacity increased in parallel with fat oxidation, reaching 2.793 μmol O₂/min/kg (±2 SE), an increase of about 50%. Mitochondrial volume density measured in biopsies of Triceps brachii and Vastus lateralis muscles also increased on the high fat diet—by 40% and 20%—providing a structural basis for increased rates of oxidation. This research was supported by grants from US and Swiss National Science Foundations, and the diets were formulated for us by the IAMS Co.

44.6 VARIATIONS IN JUMP VELOCITY IN ANURAN AMPHIBIANS: RELATIONS TO MUSCLE CONTRACTILE FUNCTION AND AEROBIC ENZYME ACTIVITY. Yu-Nei Choi and Kyungmoo Park.
Yu-Nei Choi, Washington University, Old Causeway Rd, Bedford, MA 01730.

A correlation between variation of jump velocity and variations in contractile and anaerobic capacity of the gastrocnemius muscle was examined with three anuran species, Rana nigromaculata, R. rugosa, and Bombina orientalis. Video analyses on 'maximal' take-off trials of individuals indicated that average jump velocity (m/sec) of R. nigromaculata (2.35 ± 0.17SD, n = 14) and R. rugosa (2.33 ± 0.11SD, n = 8) was significantly greater than that of the Bombina (1.74 ± 0.15SD, n = 8). Jump velocity increased significantly with decreasing tetanic rise time and with increasing rate of force production examined on the gastrocnemius muscle. Tetanic force (0.5 sec) for R. nigromaculata varied between the three species and did not correlate with jump velocity. Relative anaerobic capacity (lactate dehydrogenase activity/crimate synthase activity) of R. nigromaculata was significantly greater than that of R. rugosa and B. orientalis, while it was nearly the same between C. orientalis and B. orientalis. Thus, rate functions of hindlimb muscles may partly explain differences in anuran jump velocities, although the enzyme activity study presents obscure results to the variations. Supported by KISTEP Grant 491-4050-046-0 and Yonsei University Faculty Grant 1992 to Choi.

Bedford, MA 01730.

Relaxation after an isometric contraction is slowed by an increase in muscle length: the mechanism is not known. Josephson and Stokes (1989) reported (and most others using the work-loop method reported) that the longer running strides of large animals allow them to support the weight of the body with slower muscle fibers than the running strides of small animals. However, the distribution of muscle relaxation is much faster after working contractions than after isometric contractions. In the present study I show that frog semitendinosus relaxes faster after working contractions only at long sarcomere lengths; the difference between working and isometric contractions is negligible at the plateau of the force-length curve. Also, it relaxes faster after working contractions only at stresses from 0.06 to 0.12. At very small strains (i.e., about 20 μm/half sarcomere) it relaxes slower after working contractions.

I make two suggestions. First, I argue that, it is likely that sarcomere nonuniformities occur in vivo, especially during relaxation. Second, I argue that the nonuniformities are of biological significance; they speed relaxation.

CSF, Harvard University, Old Causeway Rd, Bedford, MA 01730.

During running some muscles stretch during the stance phase of the stride. Stretching has potential benefits in terms of enhanced generation of force and work. However, stretching active muscles beyond their short-range stiffness can also severely damage them. Recent films of running birds suggest that the muscle-tendon complex of some muscles may lengthen by 10% or more, but the distribution of this stretch between muscle and tendon is not known. Because of the potential for damage during long sprints, we hypothesized that the stretch of active muscle fascicles is normally limited to values within their short-range stiffness, about 3% of optimal fascicle length (Lg). To test this hypothesis we implanted sonomicrometer transducers in the lateral and medial gastrocnemius of wild turkeys (Meleagris gallopavo). We estimated muscle force simultaneously via strain gages glued to the bony segments of the tendons. The length-tension curve and tendon stiffness were estimated during in situ calibrations following the running trials. Birds were run on a motorized treadmill at speeds to 3.5 m/s. At speeds over 2.5 m/s, the muscles fasciculated, but this stretch was less than 3% of Lg. In addition to the confirmation of our initial hypothesis, we also discovered that during peak force production the fascicles were operating on the ascending limb of their length-tension curve. From the standpoint of economical force generation this result is surprising, because it will require a greater active cross-sectional area to generate the needed force. However, the ascending limb is an inherently self-stabilizing region on which to operate during stretch, because any sarcomeres that are stretched will become stronger.

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CSF, Harvard University, Old Causeway Rd, Bedford, MA 01730.

The mass-specific metabolic cost of running a mile (cost of transport, COT) is greater in small animals than in large ones. COT differences during running are a function of the horizontal distance the energy cost of running the body in contact with the ground (step length, L). The longer running strides of small animals allow them to support the weight of the body with slower muscle fibers that use less energy. In many animals, as in humans, COT during walking is lower than that during running. In order to understand this study to answer a simple question: does Lc also explain gait-specific differences in COT in quadrupeds? Steady state oxygen consumption was measured with an open circuit while animals walked and ran at a range of speeds. Lc was determined using high speed video. We compared small dogs (6 kg), large dogs (21 kg), goats (30 kg), and ponies (108 kg). We found that for running COT was constant across speed, but for walking COT was minimized at a moderate speed (prefered speed, duty factor 0.65). In large dogs and goats the 20% lower COT at the preferred speed corresponded to 20% lower COTs in comparison to running. In small dogs and ponies, who used the same Lc in both gait, COT did not differ between walking at the preferred speed and during running, that energetic cost is directly related to step length. This work was supported by NIH Grant ROIAR18410.

44.10 ACCLIMATION TO WEIGHTLESSNESS. Robert W. Phillips, Frank M. Suleman and Joan Vernikos.

To date no organisms have been exposed to the microgravity of space for a sufficient period of time for adaptation. However, significant acclimation occurs during brief exposures. Male rodents were flown on Space-Laboratory Life Sciences 1, in June of 1991. During the latter portion of the nine day mission animals were transported to a work station. Upon removal from the cage, animal behavior and handling ease were evaluated. It was clearly demonstrated that these rats were not neurologically damaged by the same activity in normal gravity. Weightlessness did elicit a startle reaction when the animals were floating free, but their demeanor was quiet and curious when they were restrained in the hands or could grasp other surfaces. There was no obvious decrement in their motor skills while in space. Several hours after return to Earth video recording revealed major decreases in motor function when compared to control animals. Right animals were transported again 9 days later had more normal mobility, but obvious muscle fasciculations were still present. Our conclusion is that adaptation of young rats to the space environment does not appear to have a detrimental effect on their motor skills or neuromuscular function while they remain weightless. However, the effects of space adaptation impose limits on their physical activity upon return to Earth. Further, based on the animal handling data, future missions that will require in-flight animal procedures can be confidently planned.
44.11
ONDATIVE CAPACITY AND MUSCLE MASS RESPONSES TO 14-DAY HINDLIMB SUSPENSION IN A HIBERNATOR: Endera Dababatu@, F. One Stephen@, Steven J. Wickler and Donald F. Hoyt. California State Polytechnic University, Pomona, CA 91768

Hindlimb suspension studies are used for modeling muscle disease in non-hibernators such as rats. Suspension produces muscle atrophy and decreases in oxidative capacity associated with fasting the muscle of a hibernator, the Golden-mantled ground squirrel (Spermophilus lateralis), atrophy, but oxidative capacity increases. In the present study, effects of hindlimb suspension were examined in the hibernating squirrel. Animals (collected August, 1995) were housed in cages. After 3 months, the treatment group (n=8) underwent hindlimb suspension for 14 days, while the controls (n=10) remained in their home cages. At the end of the suspension period, the hindlimbs were removed. Animals were subsequently euthanized, and hindlimb muscles were removed, stretched to resting length, and frozen for histochemistry. The remaining animals housed in undisturbed cages were used as control. The oxidative capacity in the hindlimb muscles of the treatment group was increased, while that of the control group remained unchanged. This finding suggests that hindlimb suspension can induce oxidative capacity in the hindlimb muscles of the hibernating squirrel.

44.12

One model of muscle disease is hindlimb suspension which produces both hypotension and hypoxemia. In rats, this produces greater atrophy of slow fibers than fast fibers. Our laboratory has been interested in oxidative atrophy in diurnal species that naturally undergo periods of disuse, namely, hibernators. In the current study, we examined the response of locomotor muscles in ground squirrels under hindlimb suspension. Ground squirrels (n=24) were captured in the Sierra Nevada in late July. After 3 months in the laboratory, onehalf of the animals were placed into a cage with free movement. The other half of the animals was maintained in their home cages. After 1 month, hindlimb muscles were removed, stretched to resting length, and then frozen in liquid nitrogen. Muscles studied were the plantaris, soleus, and extensor digitorum longus (EDL). Six sections were cut and stained for myosin ATPase using a pH 9.7 preincubation. Fiber types were identified by their staining characteristics and the data were analyzed using Student's t-test to compare fiber populations in hindlimb and control muscles. In addition, the oxidative fiber area ratio of each muscle was compared to its slow fiber area ratio.

44.13
IMMUNOHISTOCHEMICAL AND CYTOCHEMICAL COMPARISON OF SKELETAL MUSCLE FROM ACTIVE AND DETRAINED SQUIRRELS (Spermophilus lateralis). S. F. Evans@, D. F. Hoyt, and S. J. Wickler. California State Polytechnic University, Pomona, CA 91768

The purpose of this study was two-fold: (1) to compare immunohistochemical techniques for fiber typing with standard qualitative histochemical techniques, and (2) to determine changes in muscle fiber areas in response to a one month period of detraining. Ground squirrels (n=24) were captured in August and 12 were sampled immediately after being captured. Experimental animals (n=12) were maintained in the laboratory for one month of detraining and then sampled. Frozen muscle was cut in 8 um sections. One section was stained using MY 32 anti fast HMC for staining of fast fibers (G/A-10). The second section was stained using CS activity (EDL) and SOL; and decreased in CS activity per unit mass of tissue in EDL (21%), G/P (18%), and SOL (18%). However, there were no significant differences in CS activity/mg protein in the three skeletal muscles. 100% activity per mass unit of tissue decreased in EDL (15%) and SOL (21%). but, there was no significant difference per unit mass of tissue in G/P or per unit mass of protein in the three skeletal muscles. These results are similar to hindlimb suspension results in rats. The fact that we did not see increases in oxidative enzyme activity in CMGs may be due to season changes. Our previous detraining studies were conducted in August and September, while our current study was conducted in the middle of June. We are currently planning a similar suspension experiment in late August-early September. Supported by a NIH grant to DFH and SJW, RAP 1 R15 AR-98993-01A2.

44.14
DIOSMUS ATROIPI AND OXIDATIVE CAPACITY IN ANTELOPE GROUND SQUIRRELS, A NON-HIBERNATOR (Ammospermophilus leucurus). Steven K. Tsai, T.D. Nguyen, Steven J. Wickler and Donald F. Hoyt, California State Polytechnic University, Pomona, CA 91768

Hibernating ground squirrels reduce activity with muscle atrophy and increased oxidative capacity as seen in this phylogenetically more closely related non-hibernating species, the Antelope Ground Squirrel (AGS). AGS are summer active, hibernation being initiated in late September. Controls (n=10) were sampled immediately for carcass mass (body mass minus skin, viscera), and muscle mass of the gastrocnemius/plantaris (G/P), extensor digitorum longus (EDL), and soleus (SOL). The remaining animals were housed in rodent cages with food and water ad libitum for two months and then sampled as in the controls. The change in the EDL was not significant (p>0.05). However, the change in the G/P was significant (p<0.01). The change in the SOL was not significant (p>0.05). In addition, CS activity was not different for G/P (23.41±3 vs 25±19.9 Ug for controls and experiments, respectively) or SOL (35±1.63 vs 37±1.16 Ug). CS activity was increased in the EDL (20.6±1.3 vs 26±9.1 Ug). Reduced activity in AGS produced atrophy but did not produce a decrease in oxidative capacity as seen in more traditional models. We can suggest two hypotheses to explain these results: 1) increased oxidative capacity following decreased activity is not an adaptation to hibernation, per se; or 2) AGS has retained an ancestral capacity. This was the explanation offered by Lyman (1964, J. Mammalogy) for his observation that the proportion isolated heart of AGS was similar to the hearts of hibernators. Supported by a NIH grant to DFH & SJW, RAP 1 R15 AR-98993-01A2.

44.15
Triiodothyronine (T3) and Insulin Concentrations Associated with Two Month Normothermic Detraining in the Golden-Mantled Ground Squirrel (Spermophilus lateralis). H. Taren Tseng@, Christine S. I. Tseng@, Donald F. Hoyt, and Steven J. Wickler. Calif. State Polytechnic University, Pomona, CA 91768

A recent two-month study of reduced activity in the Golden-mantled Ground Squirrel (GMGS) reported atrophy of the gastrocnemius and increase of oxidative capacity in that muscle and in the extensor digitorum longus (EDL) (Tseng, Wickler, and Hoyt. FASEB J 8: A704, 1994). Because the latter study was conducted in the month of August when young animals maintain normal body temperatures, this state of reduced activity is referred to as normothermic detraining. The increase in oxidative capacity in these rodents is the opposite of the response in rats under similar experimental conditions. The present study was undertaken to test the hypothesis that this nonthermal response to normothermic detraining in GMGS may be due to an neuronal mechanism involving thyrotrphins and insulin. Twelve squirrels were captured in late July of 1993 and housed individually in lab cages (46 x 28 x 20 cm) with feed and water ad libitum. From July 28 to September 15, blood samples were taken at weekly intervals through the retro-orbital sinus of anesthetized, fasted animals. Plasma was collected for measurements of T3 (T3RIA, Diagnostic Products Co.) and insulin using radioimmunoassay. Data were analyzed by ANOVA with repeated measures. Both T3 and insulin levels increased during this period (T3: 0.51±0.14 to 0.9±1.0 pg/ml, p<0.05; Insulin: 3.3±1.7 to 16.2±3.1 pg/mU/ml, p<0.01). These elevated levels suggest that T3 and insulin may be significant for maintaining oxidative integrity in the skeletal muscles of this rodent species. Supported by an NIH grant to DFH & SJW (RAF151SR59893-01A2).

44.16
KINETIC ADJUSTMENTS OF AN INTERMEDIATE CRAB LOCOMOTORY IN AQUATIC AND TERRESTRIAL ENVIRONMENTS. Marlene M. Martinez* and Robert J. Full, Univ of Calif, Berkeley 94720

The buoyant force in water causes a 1/6-1/10 fold decrease in effective weight as a crab moves from air to water. Simulated reduced gravity experiments were used to predict modifications to the mechanics of aquatic versus terrestrial crab locomotion. We determined 3-D locomotory kinematics of 34 protiom of the intertidal crab (Grapsus grapsus) as it moved over a flat substratum through air and water. Air and water trials at a mean speed of 6.0 cm/s were analyzed for six crabs. Crabs moving under water showed the predicted result of a decreased duty factor compared to locomotion in air, but did not show a decreased stride frequency or increased stride length. Fewer legs were in contact with the substratum under water, the mean stance period was 1/2-1/3 that in air. Although the height of the body was not different, the width of the crabs' carapace was 10% greater in water than air. This more stable stance still resulted in more pinching and rolling in water than in air. The angle sweep by the perox-carpus joint was also smaller under water, since most water was directed posteriorly. As predicted, the crab's ability to maintain balance in the more unstable environment required the use of more legs. The crabs used a greater number of legs at the same pair at different rates, with some legs not cycling at all throughout the strides. Striding locomotion in air versus water in fast amphipod animals such as crabs can lead to the integration of terrestrial locomotor dynamics with hydromechanics, as well as to ideas on the evolution of terrestriality.
44.17  
MULTIPLE MUSCLE KINEMATIC SIMULATION OF RUNNING ROACHES.  Anna N. Abu* and Robert J. Fall, Univ. of California, Berkeley 94720.

In an effort to address the complexity of multiple muscle systems during terrestrial locomotion, we have initiated a study of the rear leg of the cockroach, *Blaberus discoidalis*, running at 20 cm/sec. We determined the 3D exoskeletal morphology, joint kinematics and the cross-sectional areas, optimal fiber lengths, apodeme slack lengths, origins and insertions for 6 femoral extensor and 9 flexor musculo-apodeme complexes. The rear legs are the major power producing legs. Effective moment arms of extensors remained near maximum during the stance phase. However, for flexors, moment arms were maximized only the first half of the swing phase when instria of the leg is largest. Major extensors (177a, 177c) and flexors (181a, 181b) operated on the planes of their force-length curves (>60% max isometric force, Fl), whereas the smaller muscles did not (177d, 177d; 182a; 44-100% Fl). Because cockroach musculo-apodeme systems are considered to be stiff (0.07-0.05); apodeme slack length to optimal muscle fiber ratio), we were able to estimate muscle fiber velocities. Extensors maintain a constant velocity (0.5-9.5 cm/sec) during the stance phase. Flexor velocities (4.16-1.6 cm/sec) peaked one third into the swing phase. Results for major rear leg muscles are consistent with economical power production. We do not anticipate these findings for the other legs. 

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44.18  

We do not anticipate these findings for the other legs.

In an effort to address the complexity of multiple muscle systems during terrestrial locomotion, we have initiated a study of the rear leg of the cockroach, *Blaberus discoidalis*, running at 20 cm/sec. We determined the 3D exoskeletal morphology, joint kinematics and the cross-sectional areas, optimal fiber lengths, apodeme slack lengths, origins and insertions for 6 femoral extensor and 9 flexor musculo-apodeme complexes. The rear legs are the major power producing legs. Effective moment arms of extensors remained near maximum during the stance phase. However, for flexors, moment arms were maximized only the first half of the swing phase when instria of the leg is largest. Major extensors (177a, 177c) and flexors (181a, 181b) operated on the planes of their force-length curves (>60% max isometric force, Fl), whereas the smaller muscles did not (177d, 177d; 182a; 44-100% Fl). Because cockroach musculo-apodeme systems are considered to be stiff (0.07-0.05); apodeme slack length to optimal muscle fiber ratio), we were able to estimate muscle fiber velocities. Extensors maintain a constant velocity (0.5-9.5 cm/sec) during the stance phase. Flexor velocities (4.16-1.6 cm/sec) peaked one third into the swing phase. Results for major rear leg muscles are consistent with economical power production. We do not anticipate these findings for the other legs. 

NSF Grant FY1 DCB 90-35138.

44.20  
ADAPTATION TO PHYSICAL WORKLOAD IN ELDERLY SPORTSMEN. Sergey V. Korobelnikov Institute of Gerontology, Kiev, 254114, Ukraine.

The study involving two groups (group 1 - 30 sportsmen aged 18-28; group 2 - 16 sportsmen aged 42-55, respectively), was undertaken to assess the physiological adaptability of peak force in response to physical training.

The physical load increasing two step-wise: 50 watt and 100 watt, during 6 min. The following were registered: heart rate, blood pressure, muscle force, muscle endurance, physical working capacity (PWC) and functional capacity of heart endurance. The data showed that the 42 % of young sportsmen and 20 % of elderly sportsmen has high level of the PWC. At the same time, 47 % of young sportsmen and 40 % of elderly sportsmen had high level of the FHR. This demonstrate an age-related decrease of the physical working capacity and functional capacity of heart endurance of elderly sportsmen. In other words, the muscle force and endurance decrease progressively with increasing age. The adaptation on physical workload in elderly sportsmen characterized by the mobilization of functional reserves of the heart.

PLENARY SESSIONS FORUM  WEDNESDAY

54.1  
IS LACTATIONAL PERFORMANCE LIMITED BY THE ABILITY TO EAT ENOUGH FOOD, OR THE ABILITY TO MAKE ENOUGH MILK? Kimberly A. Hammond and Naomi Fishbein, UC Irvine, CA 92618.

Hammond and Jared Diamond UCLA, Los Angeles, Ca. 90024.

In an effort to address the complexity of multiple muscle systems during terrestrial locomotion, we have initiated a study of the rear leg of the cockroach, *Blaberus discoidalis*, running at 20 cm/sec. We determined the 3D exoskeletal morphology, joint kinematics and the cross-sectional areas, optimal fiber lengths, apodeme slack lengths, origins and insertions for 6 femoral extensor and 9 flexor musculo-apodeme complexes. The rear legs are the major power producing legs. Effective moment arms of extensors remained near maximum during the stance phase. However, for flexors, moment arms were maximized only the first half of the swing phase when instria of the leg is largest. Major extensors (177a, 177c) and flexors (181a, 181b) operated on the planes of their force-length curves (>60% max isometric force, Fl), whereas the smaller muscles did not (177d, 177d; 182a; 44-100% Fl). Because cockroach musculo-apodeme systems are considered to be stiff (0.07-0.05); apodeme slack length to optimal muscle fiber ratio), we were able to estimate muscle fiber velocities. Extensors maintain a constant velocity (0.5-9.5 cm/sec) during the stance phase. Flexor velocities (4.16-1.6 cm/sec) peaked one third into the swing phase. Results for major rear leg muscles are consistent with economical power production. We do not anticipate these findings for the other legs. 

NSF Grant FY1 DCB 90-35138.

54.2  
QUALITATIVE AND QUANTITATIVE STRATEGIES OF THERMAL ADAPTATION OF GRASS CARP (Ctenopharyngodon Idella) CYTOLAPMIC MALATE DEHYDOGENASES.  Jen-jen Lin, Shirley Mackieol*, Ing-Nan Chen*, and Chien-ming Koo*

National Taiwan Univ., Inst. of Fisheries Science, Taipei, Taiwan 10764, ROC.

Cytolapmic malate dehydrogenase (mMDH) is involved in gluconeogenesis, lipogenesis and malate-nitrate shuttle. These important metabolic processes may likely involve the metabolic reorganization during temperature adaptation. Grass carp (Ctenopharyngodon idella), as other fishes, have two gene loci characteristics of the two isozymes partially purified from white muscle showed that the half life of thermostable mMDH at 42°C is two order higher then that of the thermolabile form. The thermostable mMDH was more sensitive to the inhibition of malate, while the thermolabile mMDH is more sensitive to the inhibition of oxaloacetate. The apparent Michaelis Menten constant (Km) of NADH measured at 20°C (pH 7.5) for the thermolabile mMDH was more then twice than that of the thermostable mMDH. In contrast, the Km value of OAA for the thermolabile mMDH was about one third that of the thermostable mMDH. Furthermore, temperature acclimation of grass carp showed that white muscle mMDH activity appeared in higher concentrations in white muscle of 11°C-acclimated fish, while the thermolabile form was more abundant in the 30°C-acclimated fish. Total MDH activity in 11°C-acclimated fish was about twice that of the 30°C-acclimated group. These results suggested that temperature acclimation could induce temperature compensation in MDH activity and differential expression of thermostable and thermolabile mMDH isozymes.

54.3  

We explored the hypothesis that maximum speed is limited by the maximum mechanical power output of the muscular system. We systematically varied the mechanical work required to run a unit distance by changing the area of the running surface (20", 40") and we measured the effect on maximum speed and mechanical power output in two lizard species (Coleonyx variegatus and Eumeces skiltonianus; body mass = 4.5g, body temp 35°C). Force production was measured while the power output of the center of mass (PCOM) during sprinting on a level ground was approximately 1 W kg-1. For both species, PCOM was dominated by the horizontal (anterior-posterior) component which was approximately 10 times greater during sprinting than during level walking. We measured an increase of the maximum speed up a 40" incline, the mechanical power required to lift the center of mass up the hill was approximately 1 W kg-1 greater than PCOM during level sprinting. In addition, to examine whether maximum stride frequency rather than maximum power output limits maximum running speed, we compared the stride frequency used during level and uphill sprinting. The lizards increased speed by increasing both stride frequency and stride length, and they used a similar stride frequency at a given speed regardless of slope. The stride frequency at maximum speed was similar on the level and on a 40" incline. We conclude that the capacity of the high muscular system to deliver mechanical power does not limit maximum speed. Supported by NIH AR01819.
WEDNESDAY
PLENARY SESSIONS FORUM
A-87

34.3
THE ENERGETICALLY OPTIMUM PATH IN GRADIENT LOCOMOTION
Albert P. Moore*, Dept. of Muscle Physiology - Istituto Tecnologie Biomediche Avanotta - C.N.R., Via Amore 56, 20131 Milano, Italy


Crystallization temperature, freeze tolerance, ice content, and plasma glucose were measured in wood frogs (B. sylvatica) from western Pennsylvania during the spring, summer, and autumn. The crystallization temperature of wood frogs (ca. -3°C) was not dependent on their body mass, and it was largely unaffected by seasonal conditioning. Autumn-collected frogs survived freezing at a lower temperature than spring-collected frogs, indicating that the cold hardiness of these animals is largely independent of seasonal conditioning.

34.4
EVIDENCE AGAINST A DISCRETE PEAK FOR AN OPTIMUM HEMATOCRIT FOR MAXIMUM OXYGEN UPTAKE (VO2max) AND FOR MAXIMUM PROLONGED SWIMMING (U090) IN RAINBOW TROUT. Anthony P. Farrell and Patricia E. Gallagher, Simon Fraser University, Burnaby, B.C. Canada.

This study challenges the idea that a discrete optimum hematocrit (Hct) exists and that this represents a trade-off between arterial O2 carrying capacity and blood viscosity as it affects cardiac work. Since the greatest demand placed on the cardiovascular system occurs during exercise, we tested the idea of an optimum Hct by swimming experimentally induced anemic, normocytic and polycythemic trout at 1°C while measuring various cardiorespiratory variables, including O2 transport (To2). A Hct of 27% was found to be normocytic, but Hct varied between 23% and 33% for individual fish. Anemia (Hct <22%) reduced To2 by 15% and VO2max was higher than the normocytic and polycythemic range for Hct (23%-55%). To2 was still proportional to Hct (as in anemic fish), but the gains in VO2max per increment in Hct were less pronounced. There was no peak value for VO2max. Furthermore, the peak VO2max occurred at an Hct of 27%, a value below the normocytic range. Similar relationships between VO2max and V02max versus Hct were observed in experiments where temperature was lowered to 5°C to increase blood viscosity. Therefore, our data provide strong evidence against the idea of an optimum Hct for swimming salmonids.

We suggest that Hct levels are better interpreted in terms of perfusion versus diffusion limitations. Work funded by NSERC Canada.

55.1
SEASONAL VARIATION IN THE CYTOBIOLOGY OF WOOD FROGS FROM PENNSYLVANIA. Jack R. Lavine, Jr. Slippery Rock University, Slippery Rock, PA.

Crystallization temperature, freeze tolerance, ice content, and plasma glucose were measured in wood frogs (B. sylvatica) from western Pennsylvania during the spring, summer, and autumn. The crystallization temperature of wood frogs (ca. -3°C) was not dependent on their body mass, and it was largely unaffected by seasonal conditioning. Autumn-collected frogs survived freezing at a lower temperature than spring-collected frogs, indicating that the cold hardiness of these animals is largely independent of seasonal conditioning.

55.2
COLD- AND DIET-INDUCED ACTIVATION OF 1 A 4-DIETARATE IN CARR LIVER AND ISOLATED HEPATOCYTES. A. Martinez, P. Tris and A.L. Cosma, Department of Environmental and Evolutionary Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK.

The most consistent cellular response to cold exposure was an increase in lipid unsaturation. The expression of an enzyme which may be centrally involved in modifying the unsaturation of the fatty acid pool, the hepatic desaturase, has been monitored following chronic cooling of carp. This was achieved by measurements of enzymatic activity, desaturase protein levels by Western immunoblotting and levels of desaturase mRNA by Northern analysis. A three day cooling regime imposed on 30°C-acclimated fish led to a 20-fold increase in activity. Surprisingly this matched an increase in immunodetectable protein. Desaturase mRNA was not detected in warm-acclimated carp but was evident after 2 days of cooling. These preliminary results indicate that desaturase induction is, at least in part, transcriptional activation. Levels of desaturase have also been measured in cultured hepatocytes from warm-acclimated carp. Lature at 30°C for up to 4 days led to an increase in activity which was again linked to increases in protein levels and mRNA production. Cooling of cells did not lead to any increase in activity over that observed at 30°C indicating a lack of cold-induction in vitro. This contrasts with cold-induction in vivo and suggests that either the conditions in culture prevented the normal cold response or that it is stimulated by some systemic influence (Supported by N.E.R.C.).

56.1
PROTEIN SYNTHESIS RATES DECREASE AFTER TWO HOURS OF ANOXIA IN ISOLATED PERFUSED TURTLE HEARTS. M. Driese and J. Bailey4, Mount Allison University, Sackville, New Brunswick, Canada, B4 J0L 2S0.

Protein synthesis, as measured by 3H-phenylalanine incorporation, was studied under conditions of normoxia and anoxia in isolated perfused turtle (Chrysemys picta) hearts at 15°C. Heart rate, cardiac output, and ventricle pressure development were unaffected by two hours of anoxia. Protein synthesis rates in ventricle were lower under conditions of anoxia (0.11 nmole HR/g protein/hr) than normoxia (0.46 n mole HR/g protein/hr). Mitochondrial protein synthesis rates decreased and the ratio of mitochondrial to total protein synthesis was lower after acute ischemic myocardial perfusion. Citrate synthase activity also declined (normoxia 0.27; anoxia 0.12 nmole/g protein/hr). Tanned mitochondria were still coupled following two hours of anoxia. Despite the anoxia, energy levels in the heart were presumably still high as contractility was maintained. The decrease in protein synthesis may not be attributable to a decrease in energy turnover but rather some other factor such as a decrease in pH. The study also reveals that the turtle heart is a good model system to study the effects of anoxia on protein synthesis without the potentially confounding factor of contractile failure.

Supported by New Brunswick Heart & Stroke Foundation and N.F.E.R.C. of Canada.

56.2
ANOXIA-INDUCED INCREASES IN CEREBRAL BLOOD FLOW IN TURTLES: ROLE OF ENDOXAN. Patrick Hyland1, Odan E. Nilsson1 and Peter L. Lutx2, VertebZebra Physiology and Behaviour Unit, Dept. Limnology, Uppsala University, Norbyuvgen 20, S-752 36 Uppsala, SWEDEN and 2 Florida Atlantic University, Boca Raton, Florida 33431-0991, U.S.A.

The freshwater turtle (Trachemys scripta) survives anoxia for days at room temperature and months close to O°C. In vertebrates, swimming experiments have indicated that the rate would probably demand a speed independent, Hct (as in anemic fish), but the gains in VO2max per increment in Hct were less pronounced. There was no peak value for VO2max. Furthermore, the peak VO2max occurred at an Hct of 27%, a value below the normocytic range. Similar relationships between VO2max and V02max versus Hct were observed in experiments where temperature was lowered to 5°C to increase blood viscosity. Therefore, our data provide strong evidence against the idea of an optimum Hct for swimming salmonids.

We suggest that Hct levels are better interpreted in terms of perfusion versus diffusion limitations. Work funded by NSERC Canada.

ADAPTATIONS TO HIGH AND LOW OXYGEN STRESS
OUTFLOW OF K+ IN TELECEPHALON OF CRUCIAN CARP AND RAINBOW TROUT DURING ANOXIA AND OXYGEN DEFICIENCY—MAY INFLUENCE SENSITIVE K+ CHANNELS

K. D. Ljubanauckas and E. N. Vinogradov. Institute of Limnology, Uppsala University, Box 580, S-752 36 Uppsala, SWEDEN

The crucian carp (Carassius carassius) is one of the most anoxic-tolerant vertebrates known. By using anodic electrolysis with carbon as anode product, the crucian carp is able to maintain ATP-levels and ionone in analysing the brain tissues in anoxia. The crucian carp (Oncorhynchus mykiss), on the other hand, is very sensitive to low temperatures. In oxygen availability, it can rapidly die in anoxia. In our results, we have observed that the ATP-sensitive K+ channels (ATP channels) are of great importance for the stability of the extracellular K+ in neurons during anoxia. Thus, experiments on rat brain during anoxia have shown that extracellular K+ levels (EKT) rise more slowly when these channels have been blocked by the ATP channel blocker glibenclamide (Jiang et al., J. Physiol. 448, 599-612, 1992).

By superfusing the brains of crucian carp and rainbow trout with glibenclamide and monitoring [K+]o with ion selective microelectrodes, we have examined the possible roles of these channels in [K+]o release during anoxia (rainbow trout) or during Na+/K+ pump blockage with ouabain (crucian carp). The results show that there is no difference in the rate of K+ outflow between tissue treated with glibenclamide and controls. This indicates that other mechanisms than KATP channels are responsible for the rise in [K+]o in fish brains during energy deficiency or Na+/K+ pump inhibition.

TEMPERATURE AND BLOOD FLOW IN PIGEON BRAIN DURING HEAT AND HYPOXIA

Marvin H. Bernstein, Shawn D. Pierce*, and Barry Darkness. New Mexico State University, Las Cruces, NM 88003

Birds regulate the temperature of the brain (TB) and of the body core (Tb) independently. They do this by adjusting the fraction of cerebral blood flow (CBF) arriving via vascular heat exchangers that cool arterial blood and thus the brain. To obtain information about the regulation of TB and CBF, pigeons (Columbia livia, mass 3.5 kg) were kept thermonutral or were heated, and were given normoxic air (PO2 138 Torr) or N2-diluted air (PO2 55 Torr). TB were measured with thermocouples in the colon and hypothalamus and estimated relative CBF from H2-washout rates, correlated with a Pt electrode in the hypothalamus. During heat stress, CBF increased significantly, but during simultaneous hypoxia and heat stress alone, TB and Tb increased, but so did the difference (AT) between Tb and Tb. During hypoxia AT was always decreased, from about 2.5°C to about 1.5°C. In thermoneutral hypoxic birds this was due to a fall in Tb while Tb remained unchanged. In heat-stressed hypoxic birds it was due to a rise in Tb toward an unchanging Tb. During either hypoxia or heat stress, CBF increased significantly, but during simultaneous hypoxia and heat stress CBF did not change. Thus, hypoxic, thermoneutral pigeons kept the temperature of cerebral blood constant but increased its flow, whereas hypoxic, hyperthermic pigeons allowed cerebral blood temperature to rise but did not change its flow. The effect on brain O2 supply awaits further study. (Supported by NSF grant R8080666.)

SULFIDE OXIDATION IN THE MITOCHONDRIA OF ARENICOLA MARINA. Guusane Völkert and Manfred K. Grieshaber, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany

The lugworm Arenicola marina inhabits intertidal sediments which can be rich in sulfide. In the presence of oxygen sulfide entering the body of A. marina is detoxified by its oxidation to thiosulfate (Völkert, S., Grieshaber, M.K. (1992) J. Comp. Physiol. 162, 469). Sulfide oxidation was localized in the mitochondria of A. marina. The addition of sulfide to isolated mitochondria caused an enhanced oxygen consumption which followed a Michaelis Menten kinetic (Völkert, S., Grieshaber, M.K. (1994) Mar. Biol. 118, 137). Sulfide oxidation was associated with ATP production and with an equimolar production of thiosulfate. Sulfide oxidation was inhibited by Antimycin and by salicyl hydroxamic acid but not by Rotenon or high concentrations of sulfide. Sulfide oxidation was performed less sensitive to cyanide than respiration with succinate as the only substrate. This data indicate that mitochondrial sulfide oxidation in A. marina is linked to the respiratory electron transport chain. At high internal sulfide concentrations electrons arising from sulfide oxidation are supposed to be transferred to an alternative terminal oxidase.

TECHNOLOGY AND ENERGETICS

56.1

TEACHING PRINCIPLES OF ANIMAL BEHAVIOR AND PHYSIOLOGY WITH A TEMPERATURE GRADIENT. C. B. McConnell and H. L. GRABENSTEIN. Biology dept., Portland State Univ., Portland OR 97207

All animals seek to maximize survival and optimize fitness. This is achieved by manipulating the body temperature. To achieve this, all mobile animals rely upon thermoregulation which can be observed, quantified, and measured in a laboratory temperature gradient. Preferred temperature may vary with life stage, as depicted by tadpoles in metamorphosis. Altered temperature selection can aid survival in low oxygen environments, as demonstrated by the common goldfish, which responds to hypoxia by lowering body temperature. Point for body temperature of mammals may be lowered (i.e. by injection of the drug ethanol) or raised (i.e. by injection of pyrogens). An alteration in set point can be clearly exhibited using behaviorally thermoregulating mice. Temperature gradients can be constructed simply and economically. They offer students an opportunity to observe thermoregulatory behavior, and to quantify behavioral thermoregulatory responses to physiological perturbations.

56.2

NATURAL POTENTIAL BODY TEMPERATURES OF NON-ADULT DROSOPHILA MELANOGASTER IN RELATION TO HEAT-SHOCK PROTEIN EXPRESSION. J. D. Newland, C. T. Barr*, and Hunter Figure*., Univ. of Chicago, Chicago, IL 60637.

Although Drosophila melanogaster is a major experimental model for the heat-shock response in Drosophila, little is known about the temperature regime of this species and thus the physiological relevance of its heat-shock response is unclear. Accordingly, we characterized variation in temperatures of rotting fruit, probably the natural habitat of this species. The results of this study show that temperature is the most important factor for the survival of Drosophila melanogaster. Worms were placed in artificial burrows in differential respirometers and exposed to ambient PO2 between 25 and 300 mmHg. At normoxia, over 60% of CBF uptake occurs across the body wall, with the remainder occurring across the hindgut. At 0.25 mmHg, total O2 consumption decreases by 50% O2 uptake across the body wall decreases by 50% over the same range, whereas hindgut O2 uptake remains constant. Thus, as ambient PO2 decreases, the average contribution of the hindgut to total O2 consumption increases from 17% at 300 mmHg, to 36% at normoxia, and to 50% at hypoxia. This increase in O2 uptake by the hindgut is achieved by an increase in ventilation rate, which is due primarily to a greater volume per ventilation. Thus, U. caupo supplements passive O2 uptake across the body wall by increasing ventilation and, therefore, O2 uptake at the hindgut during hypoxia.

REGULATION OF OXYGEN UPTAKE ACROSS TWO RESPIRATORY SURFACES IN THE MARINE WORM, URECHIS CAUPO. Wendy E. Pessac, David Julian and Alisa J. Arp. San Francisco State University, S.F., CA 94132

The echinur worm Urechis caupo inhabits intertidal mudflats along the central California coast where tidal cycles expose it to ambient PO2's below 50 mmHg. To determine the mechanisms by which U. caupo maintains O2 consumption in hypoxic water, we examined the regulation of O2 uptake across the two respiratory surfaces: the muscular body wall and the thin-walled hindgut. Worms were placed in artificial burrows in differential respirometers and exposed to ambient PO2's between 25 and 300 mmHg. At normoxia, over 60% of O2 uptake occurs across the body wall, with the remainder occurring across the hindgut. At 0.25 mmHg, total O2 consumption decreases by 50% O2 uptake across the body wall decreases by 50% over the same range, whereas hindgut O2 uptake remains constant. Thus, as ambient PO2 decreases, the average contribution of the hindgut to total O2 consumption increases from 17% at 300 mmHg, to 36% at normoxia, and to 50% at hypoxia. This increase in O2 uptake by the hindgut is achieved by an increase in ventilation rate, which is due primarily to a greater volume per ventilation. Thus, U. caupo supplements passive O2 uptake across the body wall by increasing ventilation and, therefore, O2 uptake at the hindgut during hypoxia.

The combined effects of increased global temperatures and acid rain pose a potential threat to Canada's freshwater fisheries. To assess these concerns on juvenile rainbow trout (Oncorhynchus mykiss), a 12-week experiment was conducted in soft water (Na+ = 5747±3, Ca2+ = 97±7, µeq/l) over the period June - September 1993. Trout were exposed to combinations of the following factors (ran at 15°C, ambient temperature plus 2°C, ambient pH, and sublethal pH 5.2). Fish were fed to satiation twice daily (~2.5% body weight/day). Appetite and growth increased with increasing temperature but decreased when temperatures approached lethal levels. At acidified pH, trout exposed to low pH and better growth than trout at ambient pH. Furthermore, trout at ambient temperature showed better growth and consumed more than trout at ambient temperature plus 2°C. Routine metabolic rate were unexpectedly high in all treatments, ~65% of maximum, possibly as a consequence of the feeding regime. A second experiment was conducted over the same time period in 1994. Exposure conditions were similar but the food supply was limited to a maintenance level of ~1% of the body weight/day. The results of these two exposures will be compared, with emphasis on the influence of the feeding regimes on the response to the experimental conditions. (Supported by a NSERC Strategic Grant in Environmental Quality).


Changes in gene regulation may be important for metabolic adaptation to differing temperatures, but little is known about the mechanisms resulting in these differences. We have investigated the molecular mechanisms underlying a difference in the specific activity and concentration of LDH-B in the liver between geographically separated populations of E. heteroclitus which are exposed to substantially different environmental temperatures. These differences, which persist under laboratory conditions, are hypothesized to be due to a change in the transcriptional regulation of the Ldh-B gene (Crawford and Powers, 1992). The regulatory sequences of the Ldh-B gene were determined and their function assessed using a combination of footprinting and functional assays. There is substantial differentiation between populations in the regulatory sequences and many of these differences are located in the 250 base pairs immediately adjacent to the gene. In addition, the pattern of interaction between protein transcriptional factors and these regulatory sequences also differs between populations, as assessed using RNase I footprinting assays. Variation of this sort is likely to contribute to the differential regulation of the Ldh-B gene. This work was supported by NSF grant BSR-902648.

THERMAL ACCLIMATION MODIFIES MITOCHONDRIAL PROPERTIES. Helga Guderley & Ian A. Johnston. Gatty Marine Laboratory, Univ. of St. Andrews, St. Andrews, Scotland.

Short-tailed sculpins, Myoxocephalus scorpius, were acclimated to 5 and 15°C to evaluate the impact of acclimation temperature upon the mitochondrial sensitivity to changes in environmental temperature. Mitochondria isolated from different populations have different regulatory properties and maximum rates of substrate oxidation. Cold acclimation virtually doubled maximal rates of pyruvate oxidation at all experimental temperatures. Rates of palmitoyl carnitine oxidation were also enhanced by cold acclimation, but to a lesser degree. An approximately two-fold increase in maximum rates of substrate oxidation by the isolated mitochondria was similar. Cold acclimation of sculpins did not alter the ADP affinity of mitochondria at low temperatures but markedly increased the Kmapp values at 12.5°C and 20°C. At the acclimation temperature, mitochondrial ADP Kmapp values did not differ.
METABOLIC RESPONSES OF THE SOUTH FERNANDES* AND FERNANDO T RODRIGUES SAO SEBASTIAO, 1455 S.C. F.S.P., SP.

TEMPERATURES, WILMA R. BARRIENUEVO* AND MARLA N. DE LA CARRERA.

AERIAL TELEOSTS UNDER STRESS CONDITIONS.

Constant in normoxia and moderate with increasing temperature but at each temperature the VO2 was maintained constant over a wide range of PO2. The critical oxygen tension (PO2) for 15°C, 20°C, 25°C, 30°C, and 35°C, respectively. Gill ventilation (Vg) increased in relationship with the temperature and hypoxia as a result of an accentuated increase in breath frequency (fp) and breathing volume (Vb) in and decreased in very high hypoxia levels at 35°C. Oxygen extraction was kept constant in normoxia and moderate hypoxia (PO2 > 70) regardless of the temperature. P. scrofa showed high tolerance to hypoxia under different temperatures although this effective performance could be limited by the capacity of ventilatory mechanisms to alleviate hypoxic stress under higher temperature levels.

OXIDATIVE FUEL METABOLISM OF THE VIRGINIA OPOSSUM IN PROLONGED EXERCISE AND FASTING. JERROLD W. WEIGLE.

D. L. O'Connell, Biology, University of Ottawa, Ontario, Canada KIN 6NS

Rates of oxygen consumption (VO2), CO2 production (VCO2) and urea nitrogen excretion (UN) were measured in adult Virginia opossums (Didelphis virginiana) to quantify their relative use of carbohydrates, lipids and proteins. Gas exchange measurements were carried out during low-intensity treadmill exercise lasting 2 h, and in animals living in a respirator for 6 days (3 days/tasted 3 days), while urine was collected daily. During exercise, protein oxidation only played a minor role (<6% VO2). At the onset of work, more than 90% of the energy came from the oxidation of small carbohydrates reserves. After 30 minutes, carbohydrates oxidation declined rapidly with high lipid oxidation concurrent until the utilization of these 2 fuels became equal towards the end of exercise. In resting animals, protein oxidation accounted for 18% of VO2, and this value was not changed by fasting. In contrast, a 3-day fast caused a steady increase in lipid oxidation (5% to 7% VO2) and a major decline in carbohydrate oxidation (50% to 6% VO2). We conclude that the opossum can protect its limited carbohydrate reserves during fasting by increasing lipid and maintaining protein oxidation. However, unlike most mammals, it is incapable of performing prolonged, low-intensity exercise without using carbohydrates at high rates. Supported by NSERC, Canada to J.-M. W.

OXYGEN CONSUMPTION, BODY TEMPERATURE AND VENTILATION IN BLACK-CAPPED CHICKADEES DURING ACUTE COLD STRESS. DANIEL T. CLELAND.

Department of Biology, Williams College, Williamstown, MA 01267.

Black-capped chickadees were exposed to short bouts of severe cold stress, using a 75% helium/25% oxygen (HeO2) gas mixture in an open-circuit respirometry system. Peak rates of oxygen consumption (Vo2max) in HeO2 were determined by lowering ambient temperature (T3) in discrete stages from 1°C to VO2max in HeO2 were followed by recovery in air. Body temperature (Tb) was measured continuously by intraperitoneal thermocouple, and ventilatory frequency (f ) was measured by body plethysmography. Each bird in HeO2 was measured down to Ta above which Tbmax and Tb began to decline rapidly. Standard Vg and Vtg in air were measured in late summer, cold stress measurements in HeO2 were done in early fall and mid winter.

Tbmax in HeO2 averaged 4.8 times the standard Vg, and did not differ significantly between factors, neither between the two bouts of severe cold stress. Peak Tg in air were measured down to Ta above which Tb began to decline rapidly. Standard Vg and Vtg in air were measured in late summer, cold stress measurements in HeO2 were done in early fall and mid winter.

These findings suggest that reduction of thermal conductance, rather than increased metabolic rate, is important for seasonal cold adaptation of this species. Furthermore, the low Q10 of this species could be important in maintaining arousal from hoard hypothermia at very low Ta.


A closed-circuit respirometry system was used to study biomechanical and metabolic power of unrestrained diving lesser scaup. Antirhody nis. Mechanical power output and aerobic power input (oxygen consumption) were 5.800 and 2.840 W/kg, respectively. Buoyancy contributed 62% of the mechanical cost of descent and 87% of the cost of staying at the bottom while feeding. Drag forces contributed 27% and 13%, and inertial forces due to new acceleration contributed 11% during descent and 0% (assumed) during the feeding phase. Buoyancy caused by air in the respiratory system and plumage fall from 6.2±0.4 N kg̅1 to 4.2±0.3 N kg̅1 during the dive due to air loss from the plumage and hydrostatic compression of the remaining air. Hence, these gas compartments greatly influence both the quantity of oxygen stored and the rate at which it is used during breath-hold dives: respiratory system affects buoyancy and oxygen store, plumage affects buoyancy and thermal insulation. Incorporation of inert gas washout analysis into the respirometry technique has enabled separate quantification of respiratory and plumage gas volumes. Preliminary data indicate that the volumes vary according to experimental conditions suggesting that diving ducks may regulate these gas volumes during foraging. Supported by NSERC Canada.
57.15 THE ENERGETICS OF ACCELERATED OVARY DEVELOPMENT IN ARCTIC QUEEN BUMBLEBEES. F. Daniel Vogt, State Univ. of N.Y., Plattsburgh 12901. Bernd Heinrich, Univ. of Vermont, Burlington 05405.

Arctic queen bumblebees maintain higher abdominal temperature (Tab) than temperate queens when foraging and nest-hunting soon after they emerge from hibernation in the spring. Elevated Tab accelerates ovary development and colony-founding thereby allowing arctic bumblebees to establish colonies during the short arctic summer. The energy required to maintain high Tab is in part determined by insulation. We determined the insulating capacity of bumblebee queens. In general, arctic (from Alaska) queen bumblebees had lower abdominal insulation than temperate queens (from northeast U.S.). These results reinforce our interpretation of abdominal incubation by pregnant arctic bumblebee queens as an adaptive response to their environment. This research was supported in part by NSF (grant no: BSR-9106930, Camp Denali, Alaska, and the North Slope Borough Dept. of Wildlife Management, Alaska).

57.16 EFFECT OF COLD-ACCLIMATION ON CAPILLARITY AND FIBER ULTRASTRUCUTURE IN PECTORALIS MUSCLE OF PIGEON. Q. Mathieu-Costello, P.L. Avery, K. Rosney and M.H. Bernstein, Dept. of Medicine, UCSF, La Jolla CA 92039-0623, and Dept. of Biology, New-Mexico State Univ., Las Cruces, NM 88003-0007.

The structural adaptation of muscle to exposure to cold is important to the understanding of muscle plasticity at altitude, since cold stresses are often concomitant to the exposure to hypoxia. We examined capillary-fiber structure in pectoralis muscle of 4 king pigeons (Columbia livia; BW, 780 ± 36 (SE)g) kept at 0-2°C for 60 days. The volume fraction of lipid droplets in aerobic fibers was 4-fold greater in cold-exposed pigeons (group mean, 7.8 ± 0.5%), compared to normothermic sedentary (2.7 ± 0.6%) or wild-cought pigeons (1.7 ± 0.6%), while the size and distribution of aerobic and glycolytic fibers, fiber mitochondrial density, capillary density and geometry were unchanged. Capillary surface density and intrafiber volume fraction of lipid droplets, as well as fiber mitochondrial density, capillarity and lipid droplets were all closely correlated in cold-exposed pigeons. The results suggest that the closely matched aerobic capacity and vascularization of the heavily oxidative fibers in pectoralis muscle of pigeon were sufficient to cover the increased energetic demand with shivering. Chronic exposure did not alter capillary-fiber structure in the muscles. Supported by NIH POHHL7331 and NSF DEB-9707148.
58.3  
HEMODYNAMICS OF RATS AND MICE. Robyn L. Phelps, David R. Jones, and Arnold T. Mosher*. UBC, Vancouver, BC., V6T 124, CANADA

Vertebrate hemodynamics can be described by the Windkessel and Tubular models. The Windkessel model describes incident and reflected wave interactions which augment pressure but reduce flow pulsations. The Tubular model describes flow within arteries, with arterial compliance (C/L/F), and arterial length (L) determining the timing and degree of wave propagation effects. Most mammals are described as Tubular however, small species may be mismatched with regard to C/L/F. Aortic arch wave pressure and flow were measured under varying physiological conditions in rats and mice. These measurements, and other variables, were utilized to characterize their hemodynamics and C/L/F relationships. Rats can be described by the Tubular model however, over the frequency range studied, there were no discrete wave interactions in mice. Also, mice have lower mean pressures and a slower C than other mammals under similar conditions. While the cardiovascular system is characteristic of other mammalian species, it can be described as a functional Windkessel due to a C/L/F mismatch.

58.4  
CARDIOVASCULAR RESPONSES DURING INCREMENTAL HEAD-UP TILT. Marilyn Rubin, Suzanne Fortney, Stuart Lee and Linda Barrows. Saint Louis University, St. Louis, Mo. 63104 and NASA Johnson Space Center, Houston, TX. 77048

The purpose of this study was to challenge regulating mechanisms of the cardiovascular system to maintain blood pressure to the brain during incremental head-up tilt. Human subjects (N=13) were placed on a cirrocordic bed for 30 minutes in the supine position. This was followed by incremental head up tilts at 30°, 60°, and 90° positions for five minutes each. Significant (P<.05) cardiovascular changes occurred with most of the postural changes. Results of the study showed that heart rate increased progressively to its maximum at 90° head-up tilt with mean cardiac output and stroke volume decreasing progressively during this same period. Systolic blood pressure did not significantly change, however, diastolic blood pressure was significantly increased during the series of head-up tilts. Cardiovascular regulating mechanisms are challenged and adaptive during incremental postural changes.

58.5  
EFFECTS OF HEAD OUT WATER IMMERSION ON HEART RATE VARIABILITY DURING EXERCISE. R. Perini, S. Milesevic, L. Blanchard, D. Penderslagt, A. Velestinis. Inst. of Human Physiology, University of Brescia, 25123 Brescia, Italy and Dept.of Physiology, SUNY at Buffalo, Buffalo, NY.

The power spectrum of heart rate variability (HRV) was estimated by an autoregressive method at rest and during steady states of cycle exercise of increasing loads up to exhaustion in 20 °C air (A) and 30 °C water (W) in trained male swimmers (24±3yrs). It was concluded that: a) HR from resting values of 54±17/min in W and 64±14/min in A (p<.05) increased linearly with oxygen uptake (VO₂) with similar slopes both in W and A. Maximal oxygen uptake (VO₂max) was 3.7±1.1/min and 3.8±2.1/min in W and A, respectively (p<.05); b) at rest the power of low frequency component (LF, 0.05–0.15 Hz), as % of the total, was 25.8±1.4% in W and 34.9±1.1% in A (p<.05). With exercise both in W and A, LF% increased to 40.5±2% and was unchanged up to a VO₂ of 2 l/min, then decreased linearly to 0; c) the % power of high frequency component (HF, 0.15–1.0 Hz) was at rest similar in W and A (35.6±1.7%). During exercise both in W and A, HF% decreased to 10.1±0.6% up to 70% VO₂max, then increased to 23±9%. The HF central frequency increased from 0.35 Hz at rest to 0.65 Hz at VO₂max. The different blood distribution due to immersion did not affect the power spectrum of HRV at rest or during exercise. The changes observed are thus due to the readjustment in circulatory and respiratory systems and seem to depend mainly on metabolic demand.

58.6  
VASCULAR RESPONSIVENESS IN RESPONSE TO GALANIN IN THREE SPECIES OF ELASMOBRANCHS. G.P. Courtice*, E. Preston* and C. McManus*. School of Physiology & Pharmacology, University of New South Wales, Sydney, N.S.W. 2052, Australia.

The 29 amino-acid peptide, galanin, is found in perivascular sympathetic neurons in a wide range of vertebrate species. Although in eutherian mammals, galanin has either no effect on blood pressure (BP), or has a weak depressor effects, it has been shown to be a potent pressor agent in 2 species of marsupial and 1 amphibian, and causes contraction in isolated teleost vascular strips. To investigate the vasoconstrictor effects of galanin in another phylogenetic group, we tested the BP response to intravenous porcine galanin (Peninsula, 20μg/kg) in 3 species of anhysterian elasmobranchs, Heterodontus porteri, H. hassi and Rhinobatos batillatus. In addition, contraction of the isolated pancreas-mesenteric artery in an organ bath was measured in response to increasing doses of galanin (10⁻⁶M). Galanin caused a significant rise in mean arterial BP in H. porteri (P<0.01, n=6) and H. hassi but not in R. batillatus. Galanin (10⁻⁶M) caused 30-40% of the maximum K⁺ induced contraction in the isolated gut artery in all species. In R. batillatus, isolated efferent branchial arteries were tested also, but showed slight relaxation to galanin. In conclusion, galanin causes differential vasoconstriction in various vascular beds which may lead to an increase in BP in some species of elasmobranchs. (Supported by ARC)

58.7  

In glomeruli of lightly-anesthetized kangaroo capillary pressures average 0.4–0.8 cm water with a maximum value of 0.9 cm water. Average pressures measured in glomeruli isolated and perfused with filtered water-isolated. In the latter case, however, it was possible to detect two kinds of vessels whose pressure responses varied with 140%. The first type, which occupy the interior of the glomerular capillary tuft, have pressures within them that rise to a maximum of 4/5 cm water irrespective of perfusion pressures. In the second kind, which occupy the periphery of the glomerular capillary tuft, pressures rose to much higher values, at times reaching the tissue pressure. It is postulated that the response of the two kinds of glomerular capillaries form part of a mechanism for autoregulation of flow through the haftisch glomeri. 

58.8  
UPDATE ON THE COMPARATIVE PHYSIOLOGY OF PULMONARY INTRAVASCULAR MACROPHAGES. K.J. Longworth, D. McClure, A. Nikolaussen, K.A. Jarvis, R. Smith and N.C. Staub. Univ. of California, Davis, 95616 and San Francisco. 94143. The Ohio State Univ., Columbus, 43210; Univ. of Oslo, Norway.

In response to intravenous monocrotaline blue pigment particles (MB), some species retain the MB mainly in the lungs and show a rapid transient increase in pulmonary arterial (PA) pressure because they have pulmonary intravascular macrophages, evident histologically (Physiologist 29:177, 1985; PAPER J. GAL142, 1992). We have extended our studies to other species (shown in bold).

<table>
<thead>
<tr>
<th>Order/species</th>
<th>Long MB retention</th>
<th>Increase in PA pressure</th>
<th>Intravascular macrophages containing MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artiodactyla</td>
<td>Sheep, reindeer, cattle, goat</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Carnivora</td>
<td>Dog</td>
<td>N</td>
<td></td>
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<tr>
<td>Primates</td>
<td>Monkey</td>
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</tbody>
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Only species with pulmonary intravascular macrophages show an increase in PA pressure after Monocrotaline blue; the cells must be present for the response to occur (JAP 73:2608, 1992). The cat has the macrophages but does not show a change in PA pressure. Based on this data, reactive pulmonary intravascular macrophages are found only in mammals of the orders Artiodactyla and Carnivora.[Support: Amer Heart Assoc (REL) & HL25816 (Prog Proji)]]
58.3

STATIC MUSCULAR CONTRACTION ELICITS A PRESSOR REFLEX IN THE CHICKEN. T.P. Adamsen* and I.C. Solomon. Neurobiology, Physiology and Behavior and Division of Cardiovascular Medicine, University of California, Davis, CA 95616

Static muscular contraction increases arterial blood pressure and heart rate in humans and other mammals; however, it is not clear whether birds exhibit similar changes. We performed this study to determine if the chicken exhibits a pressor response to static muscular contraction, and to demonstrate that the evoked pressor response is due to a reflex arising from the contracting muscle. In 11 chloralose anesthetized cockerels, we evoked static contractions of the left gastrocnemius muscle by stimulating the left sciatic nerve for 60 seconds at 1.5-3.0 times motor threshold (30-40 Hz, 0.025 ms). We measured arterial blood pressure and muscle tension before and during static contractions, and calculated heart rate from the arterial pressure trace.

58.11


The marine bivalve, Mercenaria mercenaria, possesses an open circulatory system whereby the hemolymph is propelled through the body cavity by a three chambered heart consisting of two atria and a single ventricle. Modulation of the rate and strength of contraction has been linked to many compounds including acetylcholine (Ach), FMRFamide and 5-hydroxytryptamine (5HT). The bulbus arteriosis, and its role in cardiovascular function, remains largely uninvestigated. In this investigation, the structure and function of this organ were examined using microscopy and pharmacological techniques. Light microscopy utilizing Mallory's triple stain did not reveal the presence of intiated muscle in the bulbus arteriosis. Preliminary studies of the physiology of the organ have centered on its possible role in the regulation of cardiac contractility. Homogenates from extract bulbus arterioses were separated using a C-18 Sep-Pack cartridge and bioassayed for the presence of acetylcholine. The results suggest the presence of Ach and FMRFamide in the extract. Isolated bulbus arterioses were challenged with Ach, FMRFamide and 5HT. All three compounds (10^-6 M) induced contractions of the bulbus arterioses. Thus, we propose a possible role for the bulbus arteriosus in cardiovascular function.

58.10

ICHEMIC PRECONDITIONING IN MARMOT HEART. Tom McKean and Wade Mendenhall*. Department of Biological Sciences, University of Idaho, Moscow, ID 83844

Ischemic preconditioning (IP) is a self-protective mechanism in the heart. Hearts in which all or part of the coronary circulation has been interrupted for 20 min and reperfused immediately by means of bypass of the heart, developed far less damage than control hearts subjected to 10 min reperfusion. The damage resulting from the prolonged occlusion is markedly reduced by the preconditioning (IP) stimulus. The hypothesis of the study is that the marmot, by virtue of its fossorial habit that includes hypoxia, has greater intrinsic cardiovascular adaptability than the New Zealand rabbit. Hearts were removed from anesthetized animals and perfused with crystalloid buffer. Control hearts were subjected to 30 min global ischemia followed by 30 min reperfusion. IP hearts were subjected to 10 min of global ischemia followed by 10 min reperfusion and then 30 min ischemia and 30 min reperfusion. Heart rate, left ventricular pressure, aortic pressure and LH release were measured and compared among the different groups. The conclusions of the study are 1) IP protects the rabbits heart 2) IP probably protects the marmot heart but its effect is not as powerful as in the rabbit and 3) the marmot heart is not ischemia or hypoxia tolerant compared with the rabbit heart. This study was supported by NIH grant R16HL46601.
58.15

EFFECTS OF ARACHIDONIC ACID AND INDOMETHACIN ON TROUT HEART PERFORMANCE AND CORONARY CIRCULATION IN FISHER: Claudio Agnolin*, Tariq Mustafa, Ratu Venal, Frank B. Jenson* and Bruno Tesso Stazione Zoologica "A. D'Orbigny", 80125, Napoli, Italy. OK 320-01 Odense M, Denmark.

Arachidonic acid (AA), a common constituent of cell membranes phospholipids, is the substrate for the biosynthesis of an important group of "hormone-like" substances, the eicosanoids. AA metabolism follows two major lines, the cyclooxygenase pathway leading to prostanoid synthesis and the lipoxygenase pathway from which leukotrienes are generated. The heart and its vasculature are able to produce eicosanoids and these compounds have powerful vasoactive and myocardial actions. Eicosanoid synthesis and actions are highly species dependent. In the present work evidence for a potential role of AA metabolism in trout heart function is presented. The study was performed on isolated and perfused trout (Oncorhynchus mykiss) hearts. Two perfusion set up were used. In the first set up the trout was perfused with non recirculating saline and the coronary function in the re-circulating system, which in part reproduces the one occurring in vivo.

58.18

EFFECT OF ANOXIA AND ACIDOSIS ON INTRACELLULAR FREE Ca2+ IN ISOLATED VENTRICAL CARDIOMYOCYTES FROM TURTLES: Jutta Mar-Planck Inst. for Experimental Medicine, D-3400 Gottingen, Germany.

Cardiac inotropism is stimulated by sympathetic division α and Noradrenaline (NE). The β-adrenergic action is well established while the α adrenoreceptor mediated mechanisms are still controversial. It seems likely that α and β inotropic effect could be qualitatively different. The aim of this work is to characterize the inotropic and adaptive responses of atrium to NE. The left atrium of Wistar rats were isolated and prepared for isometric contraction recording. Two groups of male Wistar rats were submitted to swimming in one acute session (30 min, ASW, n=1) and in three consecutive days session (5, 15, 30 min. ISW, n=1). In comparison with a control group (CO, n=8). The maximum isometric force, its maximum and minimum derivative, as well as P2 value were measured response to insulin 5 and 10 U*kg-1. Insulin induced the heart rate at 10 to 12% decrease. The results of this study showed no significant different between the groups. Yet, the ASW group has shown an increased slope of dose-response curves to NE suggesting a change in effect of NE on isolated atrium in comparison to isolated hearts stimulated with unstimulated. This study was partially supported by the Max-Planck Gesellschaft.

58.19

INOTROPE ADAPTATIONS ON RATS SUBMITTED TO SWIMMING: Roseli Golfetti, Luiz E. Barreto Martins, Rosana C. Spadari and Lourenco Gallo Jr. Laboratório de Fisiologia do Exercício - PEP, Dept Fisiologia - IB - UNICAMP, Campinas, SP, Brazil, 13081-970

Cardiac inotropism is stimulated by sympathetic division α and Noradrenaline (NE). The β-adrenergic action is well established while the α adrenoreceptor mediated mechanisms are still controversial. It seems likely that α and β inotropic effect could be qualitatively different. The aim of this work is to characterize the inotropic and adaptive responses of atrium to NE. The left atrium of Wistar rats were isolated and prepared for isometric contraction recording. Two groups of male Wistar rats were submitted to swimming in one acute session (30 min, ASW, n=1) and in three consecutive days session (5, 15, 30 min. ISW, n=1). In comparison with a control group (CO, n=8). The maximum isometric force, its maximum and minimum derivative, as well as P2 value were measured response to insulin 5 and 10 U*kg-1. Insulin induced the heart rate at 10 to 12% decrease. The results of this study showed no significant different between the groups. Yet, the ASW group has shown an increased slope of dose-response curves to NE suggesting a change in effect of NE on isolated atrium in comparison to isolated hearts stimulated with unstimulated. This study was partially supported by the Max-Planck Gesellschaft.

58.16


Atrial natriuretic peptide (ANP) reduces plasma volume in mammals and circulating ANP increases cardiac blood volume in the rainbow trout (Oncorhynchus mykiss). The effect of ANP on fluid compartments in teleost fish is unknown. In this study whole body and tissue blood volumes (Ct-red cell space) and extracellular fluid volumes (Co-EDTA space) were examined after 8 h perfusion of saline (1 ml/kg) or ANP (300 ng*kg-1) in conscious rainbow trout (Oncorhynchus mykiss). ANP lowered whole body blood volume from 29.8±2.5 to 29.2±0.5 ml/kg (p<0.01; n=8) and extracellular fluid volume from 242±17 to 188±5.8 ml/kg (p<0.001; n=8). Ct-red cell space was increased (p<0.05) by ANP in gills, brain, eye, pectoral fins, ventral muscle and bullhead in decrease in cardiac index was observed in ANP increased Ct-red cell space and presented a general extracellular dehydration, whereas local ANP-mediated vaso-dilatory responses may increase blood volume in certain tissues. Supported by NIH grant R01 HL060090-01 (DD) and NS-200 grant IS 9105247 (KO).

58.20

EFFECTS OF ANOXIA AND ACIDOSIS ON INTRACELLULAR FREE Ca2+ IN ISOLATED CARDIOMYOCYTES FROM TURTLES: Jeremy S. Wasserman and Norbert Blinks. Max-Planck Inst. for Experimental Medicine, D-3400 Gottingen, Germany.

We examined the effects of anoxia and acidosis on intracellular calcium in isolated ventricular cardiomyocytes from painted turtles (Chrysemys picta bellii). These hypolipidic tolerant animals have been the subject of intensive study aimed at explaining their extraordinary ability to live without oxygen. We tested: (1) isolated cardiomyocytes that (2) exhibited normal contractility expected to elicit the cardiac cortex for SEP recording. The contractile cardiac taw was stimulated (0.2 V, 5 Hz, 0.5ms) for 70s before and during topical superfusion of 1 mM L-NA. Blood pressure was measured continuously during anoxia. After 30 min., amplitudes of both the P1 and N2 waves were reduced to 67%±18% and 55%±17% of control, respectively. After 60 min., SEP amplitudes were further reduced to 47+13% and 25±14% of control. The NO synthase substrate L-arginine partially restored SEP amplitudes. 1-NA had no effect on SEP latency. There was no change in SEPs in rats superfused with cerebrospinal fluid (+120% ±49%). These results suggest that NO inhibition most likely suppressed neuronal activity in cortex during somatosensory stimulation. (Supported by NS 21076).
59.3 OSMOREGULATION BY DRINKING IN RATS. T. Moriizuto, E. Sugimoto* and H. Nose, Dept. of Physiol., Kyoto Prefectural University of Medicine, Kitakyushu, Kyoto 602, Japan.

A method to measure circulating blood volume (BV) and plasma Na+ concentration INa+ continuously in rats was used to evaluate the hydration process from thermal dehydration. Continuous change in BV was monitored by measuring 51Cr-tagged erythrocytes dilution using an arterio-venous extracorporeal shunt passing through a gamma counter, and [Na+] was measured using a flowing-through sodium sensitive glass electrode. Thermally dehydrated rats were provided with tap water and 1.8% NaCl and cumulative amounts of tap water and 1.9% NaCl intake were recorded for 4 h together with BV and [Na+]. When both tap water and 1.8% NaCl solution were provided, the rats consumed mainly tap water in the first 45 min, and [Na+] returned to the predehydration level within 60 min. Thereafter, the rats consumed both tap water and the NaCl solution alternately, and blood volume showed gradual recovery. The results suggest that during the initial stage of rehydration, rats chose a dilute NaCl solution to reduce osmotic hydration. Later, blood volume was expanded with consumption of almost isosmotic NaCl solution. In other words, osmoregulation by drinking behavior precedes the volume regulation and volume is required for complete recovery of blood volume.

59.5 VOLUME-ACTIVATED CHLORIDE CHANNELS IN HUMAN IMMATURE TERATOMA. Meng-Ru Shen, Cheng Yang Chou, Sheng-Nan Wu, Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, Tainan, Taiwan.

The hypotonic-induced chloride channels were investigated with the whole-cell recording mode of voltage clamp technique in human immature teratoma. Exposure of the cells to hypotonic solutions visibly swelled the cells and reversibly activated an outward rectifying Cl- current, which decayed at the most depolarized voltage used. The volume-regulated Cl- current was effectively inhibited by the 100 μM 4,4'-disothiocyanostilbene-2,2'-disulfonic acid, a substance known to block Cl- channels in a variety of cells. In addition, the Cl- current can be abolished by verapamil and 1,9-dideoxyforskolin, which are known to inhibit P-glycoprotein function. Chloride current activation by hypotonicity was also dependent on the presence of ATP in the intracellular solution and this requirement could be replaced by the non-hydrolysable analogue ATPγS and Mg2+-free ATP. These characteristics of this current suggest that it is mediated by the same type of channels that have been recently associated with expression of the multidrug resistance P-glycoprotein.

59.7 REGULATORY VOLUME DECREASE (RVD) BY NECTURIS MACULOSUS RED BLOOD CELLS (RBC). D. Light, L. Bergeron*, and A. Street*, Dept. of Biology, Ripon College, Ripon, WI 54971.

The lowest osmolality (mOsm/kg H2O) RBC could be exposed to without hemolysis occurring was species dependent: Necncrus tenuilu~osus (0.01 I), Ram~erus~asteus (0.05 I), Globulus puls~ar~is (0.08 I), Equus caballi (0.140), Homo sapiens (0.150), Oris aries (0.160). Exposure of Necturus RBC to hypotonic (0.5X) Ringer's solution visibly swelled the cells and caused hemolysis. A quinine-sensitive K+ channel and a Cl- channel were observed in cell-attached patches. Conclusions: 1) cell swelling activates a quinine-sensitive K+ channel, 2) this channel mediates K+ loss during RVD, and 3) channel activation is mediated, at least in part, by a phosphorylation-dependent mechanism. (Supported by NSF grant MCB-9304247.)

59.8 ARGinine vasopressin (AVP) IS INVOLVED IN REGULATION OF EXPRESSION OF RAT KIDNEY URREA TRANSPORTER (HT2) mRNA. Jane C. McWhinney*, Darren A. Towle, and Malcolm C. Giebisch, Medicine, The University of Chicago, Chicago, IL 60637 and Medicine, Stanford University, Stanford, CA 94305.

When water-deprived rats were exposed to arginine vasopressin (AVP), the mRNA encoding the Na+-coupled amino acid transporter (HT2) in kidney proximal tubule was increased by more than 3-fold. AVP increased mRNA at concentrations as low as 10-100 ng/ml. In addition, rUT2 mRNA has been shown to be up-regulated by AVP in whole tissue cultures, and AVP increased the expression of the Na+-coupled amino acid transporter in kidney proximal tubule. These results suggest that AVP may be involved in the regulation of the expression of the Na+-coupled amino acid transporter in kidney proximal tubule.

59.9 EFFECT OF CALCIUM CHANNEL ANTAGONISTS ON AMINO ACID RELEASE FROM BIVALVE VENTRICLES IN HYPOOSMOTIC SW. Lewis B. Deaton. University of Southwestern Louisiana, Lafayette, LA 70504

When exposed to hypoosmotic seawater (SW), the bivalve clam (Arctica islandica) antagonists on amino acid release were investigated. The AA release in 500 mOsm SW for 2 hr and the release of amino acids measured. The AA release in 500 mOsm SW was 14 and 130 nmol/g dry weight, respectively. Increasing the Ca++ concentration in the 500 mOsm SW had no effect on AA release. The AA release in 500 mOsm SW was not affected by verapamil, ionomycin, A23187, and Mg2+-free ATP. These results suggest that an influx of Ca++ ions is not involved in the control of AA release from bivalve myocellular cells in hypoosmotic SW.

59.6 ICyonic Equilibria in Shark Red Cells. J.A. Payne and T.J. Maltz. Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672. Dept. of Zoology, Univ. of Florida, Gainesville, FL 32611 and Division of Physiology, Dept. of Cell Biology, Duke University Medical Center, Durham, NC 27710.

The equilibrium distribution of permeant ions across the plasma membrane was studied in two shark species: the spiny dogfish, Squalus acanthias, a cold water form, and the rough shark, Centrolophus fasciatus, a tropical species. The AA release in isosmotic (1000 mOsm) seawater was not affected by verapamil, ionomycin, A23187, or Mg2+-free ATP. The AA release in isosmotic (1000 mOsm) seawater was not affected by verapamil, ionomycin, A23187, or Mg2+-free ATP. The AA release in hypoosmotic seawater (50 mOsm) was not affected by verapamil, ionomycin, A23187, or Mg2+-free ATP. The AA release in hypoosmotic seawater (50 mOsm) was not affected by verapamil, ionomycin, A23187, or Mg2+-free ATP. These results suggest that an influx of Ca++ ions is not involved in the control of AA release from bivalve myocellular cells in hypoosmotic SW.
59.9 VOLUME REGULATION IN TWO FRESHWATER MOLLUSCS, LAMPSILIS TERES AND POMATIA BRIDGEI, IN HYPEROSMOTIC MEDIA.

Frank J. Vourvou and Lewis R. Deaton, Dept. of Biology, University of Southwestern Louisiana, Lafayette, La 70504.

L. teres and P. bridgei were acclimated to media ranging from fresh water (50 mOsm) to 390 mOsm and from fresh water (FW) to 151 mOsm, respectively. L. teres survived in media above 200 mOsm while P. bridgei died immediately above 151 mOsm. Both animals are good volume regulators; there was no substantial decrease in tissue hydration of the gills. In L. teres gill, the saline acid content increased from 19.86 ± 2.46 umol/g dry weight (FW) to 315.75 ± 125.94 umol/g dry weight (390 mOsm). G-alanine, glycine, and alanine were the major acid pool amino acids in both gill and external osmotic concentrations. In P. bridgei gill, the acid pool increased in response to increased external osmotic concentration from FW to 100 mOsm and then decreased above 100 mOsm. The major constituents of the amino acid pool were glutamate, alanine, glycine, and threonine. These results suggest that freshwater mussels have the ability to regulate cellular volume in hyperosmotic media and that volume regulation is accomplished, in part, by increasing the levels of selected amino acids in the cytoplasm.

59.10 EFFECTS OF ACCLIMATION SALINITY ON SODIUM FLUXES IN BLUE CRABS. Gerald D. Robinson*. Towson State University, Towson, Md. 21204.

Hemolymph sodium ion (Na) concentrations and whole body Na fluxes were measured in blue crabs (Callinectes sapidus) following acclimation to artificial seawater media ranging from 0 mOsm/L to 908 mOsm/L. A marked change in Na fluxes with acclimation salinity. Apparently, blue crabs tolerate rapid bidirectional saline droplets under paraffin oil. The second approach involved perfusion of drugs in vitro with the upper and lower portions separately bathed in saline solutions. For instance, KCl reabsorption was blocked by increasing the Cl conductance of an epithelial shunt pathway. Hence, NaCl reabsorption is inhibited KCl reabsorption. A number of chloride channel blockers, including N-isothiocyanostilbene-2,2'-disulphonic acid (SITS), and Acetazolamide (an inhibitor of carbonic anhydrase), inhibited KCl reabsorption. Rapid inhibition suggests localization of Cl- in apically-sited Cl-/HCO3- exchangers. Drugs known to block Cl-/HCO3- exchangers, such as diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS), 4-acetamido-4'- diethylamino stilbene sulphonate (AEC) and 4-acetamido-4'-diethylamino stilbene sulphonate (NPPB), inhibited KCl reabsorption; rapid inhibition suggests localization of Cl- to 100 mOsm and then decreased above 100 mOsm. The major constituents of the amino acid pool were glutamate, alanine, glycine, and threonine. These results suggest that freshwater mussels have the ability to regulate cellular volume in hyperosmotic media and that volume regulation is accomplished, in part, by increasing the levels of selected amino acids in the cytoplasm.

59.11 MECHANISMS OF KCL REABSORPTION IN THE LOWER MALPIGHIAN TUBULE OF RHODNIUS PROLIXUS. Charles Haney and Michael J. O'Donnell, Department of Neurobiology, University of Washington, Seattle, WA 98195.

The Malpighian tubules of the blood-feeding insect, Rhodniurus prolixus, consist of a secretory upper portion, which transports KCl lumina1y, and a reabsorptive lower portion. Reabsorption of KCl in the lower tube can be stimulated in vitro by 37°C. Following stimulation, the lower tube can reduce luminal fluid osmolality from 370 to 250 mOsm/kg. This is primarily caused by a reduction in luminal [K+] from 24.8 ± 3.1 mmol/L to 9.8 ± 0.3 mmol/L. A significant direct correlation existed between hemolymph Na+ concentration and salinity of the acclimation medium. The crabs strongly regulated hemolymph Na+ concentrations in dilute media and were most concentrated in the most hyperosmotic media. Rates of unidirectional Na+ influx and combined flux (influx and efflux) exhibited a significant positive correlation with salinity acclimation. Apparently, blue crabs tolerate rapid bidirectional exchanges of Na+ across the body surface when they osmoconform in high salinity environments, but dramatically limit such movements during hyperosmoregulation in dilute media.

59.12 TRANSPORTER POTENTIAL DIFFERENCES AND SODIUM FLUXES ACROSS ISOLATED PERFUSED GILLS OF THE HYPER-HYPOREGULATING MANGROVE CRAB Eriocheir japonica, John B. Martinez*, Robert B. Jarvis*, and Michele W. Xu, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803. This work was supported by CAPES - Brazil and NSF 93-08143.

59.13 PEPTIDE INCREASES PARACELLULAR PERMEABILITY IN INSECT MALPIGHIAN TUBULES. Klaus W. Bubeck and Ari E. Rubenstein, Section Physiology, Cornell University, Ithaca, N.Y. 14853.

The diuretic leukocin, an octapeptide isolated from cockroach heads (Hosman et al., Comp. Biochem. Physiol. 51A: 31, 1977), dramatically reduces transepithelial voltage and resistance in isolated perfused Malpighian tubules of the yellow fever mosquito Aedes aegypti, by increasing the Cl conductance of an epithelial shunt (Pannabecker et al., J. Membrane Biol. 132: 63, 1993). To distinguish between the effect of leukocinin on transepithelial or paracellular pathways, we measured transepithelial permeabilities of inulin. The permeability to inulin was not increased by leukocinin. Inulin was excluded from movement through transepithelial pathways. Hence transepithelial permeabilities reflect the properties of an extracellular pathway such as the paracellular pathway. Inulin flux significantly increased the rate of transepithelial fluid secretion (1.8-fold) and significantly increased transepithelial inulin permeability (2.9-fold; 11 Malpighian tubules). The diuretic AMP (100 µM) also significantly increased the rate of transepithelial fluid secretion (1.59-fold) but without a change in transepithelial inulin permeability (12 tubules). These effects of AMP confirm its action on the transepithelial pathway impermeable to inulin (sawyer & beyenbach, Am. J. Physiol. 248: R339, 1985). Since transepithelial inulin permeability increases in the presence of leukocinin but not AMP, the effect of leukocinin on the paracellular pathways is demonstrated. Supported by NSF IBN 9220464.
OSMOREGULATION IN THE CRAYFISH (Procambarus clarkii): HORMONAL REGULATION BY ATRIAL NATRIURETIC PEPTIDE.


Atrial natriuretic peptide has proven to be a multi-functional hormone in mammalian systems, with natriuretic, diuretic and vasoactive actions. Mammalian systems have indicated ANP releases results in a complex cascade of interactions between the cardiovascular, renal and nervous systems. The present experiments propose a role for ANP in crustacean osmoregulation. Crayfish unidirectional ion flux was shown to be ANP dose dependant. Injects of ANP at 10^{-6} M concentration increased both Na+ and Cl- influx. Crayfish gills is activated by ANP in a dose dependant manner. Cyclic GMP present experiments propose a role for ANP in crustacean osmoregulation.


The finding that α2 is expressed in all fiber types examined indicates that all of these muscles likely participate in the homeostatic mechanisms mediated by α2/3 regulation in muscle; including the redistribution of K stores in hypokalemia, and thyroid hormone. We aimed to determine relative levels of expression of α1 and α2 in rat skeletal muscles likely participate in the homeostatic mechanisms mediated by α2/3 regulation in muscle; including the redistribution of K stores in hypokalemia, and thyroid hormone.

HYPERHYPERTHERMIC AND HYPERTONIC SHOCK INDUCE HSP70 ACCUMULATION OF RELATIVE DURATION IN THE MARINE AMPHIPOD G. pulex.

L. Fasham; Whitman College, Walla Walla, WA, USA.

Various stresses induce heat shock proteins (HSP) in most cells, including HSP70 which can protect cells from hyperthermia. Hypertonic shock induces HSP70 in cultured mammalian renal (Malpighian tubule kidney- MTK-1) cells (Duan et al., J. Exp. Med. 185: 401-412, 1997). To test if HSP70 can protect cells from osmotic shock, we exposed cells to heat shock (45°C), osmotic shock (550 mosm with added NaCl), or combination (initial shock 2.5—4°C recovery at 27°C and 300 mosm, then final shock). We judged cell survival by cell plating efficiency and/or early membrane leak. Cells grown for 24 hr with cell survival experiments; both gave similar results. HSP70 was detected by monoclonal antibody (Western blots, chemiluminescent detection). A 10 min heat shock enhanced thermotolerance recovery, and maximized HSP70 accumulation (Combination = 10 min heat shock 6 hr recovery, 35 min shock; survival results are % of controls). Our data indicate that HSP70 may be a protective mechanism in hypertonic shock.

Molecular identification of a P-type ATPase from the genome of Coccolithus pelagicus: Implications for molecular evolution and cell biology.

Tom Sone and Douglas Faulkworth. Biology Department, the Johns Hopkins University, Baltimore, Maryland 21218.

Using polymerase chain reaction amplification, we have isolated a DNA fragment from total genomic DNA of Coccolithus pelagicus (C. pelagicus), which belongs to the marine phytoplankton group Coccolithophorids. The PCR product was obtained by priming with two degenerate oligonucleotides designed against a multiple sequence alignment of known P-type ATPase sequences, with the 5' end at the phosphorylation site and the 3' end at the EF hand binding site. The new clone's sequence contains the PIP2-binding site, located in the region between the two primers. All three sites are definitive domains on the P-type cation-motive ATPases. The sequence of this coccolithophorid ATPase most resembles the protozoan and fungal intracellular Calmodulin-P+ ATPases, the mammalian P+ and secretory pathway Calmodulin-P+ ATPases, the prokaryote Mg2+ and Ca2+ ATPases and the plasma membrane P+ ATPases in fungi and higher plants. This DNA sequence is among the first P-type pumps cloned from a living organism, which may shed new light on the molecular evolutionary history of the P-type ATPases. Possibly a secretory pathway cation pump in Coccolithus may represent a substantial step towards a fuller understanding of the processes involved in cellular calcification as exemplified by this species.