Plenary Lectures and Symposia

Sunday

Monday

Tuesday

Wednesday

Poster Sessions

Sunday

Monday

Tuesday

Wednesday

1.0

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.

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NEURAL MODULATION OF MUSCLE PROPERTIES

2.1

DEVELOPMENTAL CHANGES IN PEPTIDE NEUROMODULATORS IN INSECT MOTONEURONS: IMPLICATIONS FOR THE NEUROMUSCULAR SYSTEM. J. Witten. Dept Biol Sci, Univ Wisconsin, Lapham 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 FLRFamide 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 eh 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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2.2

PEPTIDE AND AMINE MODULATION OF INSECT NEUROMUSCULAR TRANSMISSION. Michael E. Adams, Larisa D. Acevedo and David N. Mbungu. Depts. 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 cells. 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, Emory U, Atlanta, GA 30322.

Five neuropeptides terminating in the sequence RFamide have been isolated and sequenced from CNS 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 contractures 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 activity by gating on a persistent Na^+ current, modulating a voltage-gated Ca^{2+} current, and 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.

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Biophysical effects of RFamide peptides on heart muscle cells.

2.4

MODULATION AT MOLLUSCAN NEUROMUSCULAR JUNCTIONS: PHYSIOLOGICAL ROLES AND CELLULAR MECHANISMS. V. Brezina, E.C. Cropper, J. Heierhorst, W.C. Probst, F.S. Vilim, I. Kupfermann and K.R. Weiss. Dept. Physiology & Biophysics, Mt. Sinai School of Medicine, New York.

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 relaxation rate of the ACh-induced contractions. In addition to this 'intrinsic' 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 bites 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 Ca 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.

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2.5

NEURAL MODULATION OF MUSCLE PROPERTIES:
SHARING OF NEURAL AND NON-NEURAL CONTROL OF MUSCLE PROPERTIES IN MAMMALIAN SYSTEMS. V. Reggie Edgerton, Roland R. Roy and John A. Hodgson. UCLA Department 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, but the level of control has not been well-defined. The experiments of Buller and colleagues in the late '50s and early '60s demonstrated that slow skeletal muscles became 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 '80s, 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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2.6

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

The degree of atrophy exhibited in neuromuscular 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 *Barytettix psolus*, damage occuring to the leg nerve (N5) during autotomy, transneuronally induces atrophy and degeneration of myofibers of undamaged, fully innervated muscles intrinsic to the thorax. While the experimental muscles are reduced to <15% of control mass in *B. psolus*, similar treatment of related taxa, *Schistocerca americana* and *Melanoplus differentialis* causes only slight 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 *S. americana* and *M. differentialis* to an extent nearly equivalent to that in *B. psolus*, and diminishes 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 characters of *B. psolus* suggest that it is permanently juvenilized (paedomorphic) relative to its taxonomic cousins. Its 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, Jeannine Williams & Daniel Crocker
Department of Biology, University of California, Santa Cruz, CA. 95064.

Many marine mammals undergo prolonged period 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, *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 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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3.2

BIOMEDICAL LESSONS FROM THE STUDY OF APNEA IN MARINE MAMMALS. W.K. Milsom 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 apnea can be initiated by several reflex mechanisms. It's significance as a defense 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 apnea 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 pathological 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).

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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, can not 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

ASPHYXIA, ISCHEMIA AND OXYGEN RADICALS.

R. Elsner, S. Oyasaeter 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 diving asphyxia is the selective distribution of blood flow favoring organs requiring an uninterrupted supply. Other regions are exposed to prolonged and intense vasoconstriction. 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 free 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). Seal 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.

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3.6

MARINE MAMMAL ATHLETES: MODELS FOR FUNCTIONAL DIVERSITY IN MAMMALIAN LOCOMOTOR SYSTEMS.

Terrie M. Williams[1] and Randall W. Davis[2]. [1]Dept. of Biology, Univ. of CA, Santa Cruz CA 95064; [2]Texas A&M Univ., Galveston 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 contracting muscle. We've used this unique system to examine the relationships between oxygen delivery, locomotor energetics, and skeletal muscle architecture. Results for several species of pinniped and cetacean showed that fibre type composition of the propulsive muscles correlates with routine swimming speed rather than breath hold duration per se. Conversely, myoglobin concentration, which ranged from 3 to 10 gm Mb/100 gm tissue in diving mammals, showed no correlation with swimming ability. Because myoglobin has a higher affinity for oxygen than hemoglobin, average blood flow to the skeletal muscles of diving mammals must be restricted by 88% of resting to fully utilize these muscle oxygen stores. Thus, a paradoxical decrease in muscle blood flow is required when the metabolic demands of the skeletal muscle are increased during aerobic dives. These studies on marine mammals demonstrate the plasticity of the circulatory and skeletal muscle systems when oxygen delivery is limited.

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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.

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EVOLUTION OF ENDOTHERMIC 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 indicate 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 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.

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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 3-5x "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- and endotherms. Therefore the activity of each sodium pump ie the molecular activity has increased several fold during the evolution of endothermy. At the same temperature (37°C) sodium pumps from ectotherms can each turnover 2,500 ATP/min compared to 9,000 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.

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4.5

Mitochondrial function and endothermic metabolism

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

The bearded dragon, *Amphibolurus 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 then 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 than twice as fast as lizard liver mitochondria. In both species 15% of hepatocyte respiration is non-mitochondrial, about 25% drives a futile cycle of proton pumping and proton leak across the mitochondrial inner membrane and about 60% is used to drive ATP synthesis, thus all three processes run more than twice as fast in rat liver cells as in lizard cells (2). Increased non-mitochondrial oxygen consumption and ATP synthesis could be caused by increased enzyme activities, but what causes the increased proton leak rate? Rat liver mitochondria are four- to fivefold leakier to protons but probably operate at lower potential in situ, resulting in a more than doubled proton leak rate. This increased leakiness is linked to a greater unsaturation of phospholipid fatty acids (2). Phospholipids from rat liver mitochondria form vesicles that appear to be leakier to protons than those from lizards, and the proton leakiness of the vesicles correlates with unsaturation index (3). Thus as well as increased ATP turnover, endothermy involves a proliferation of mitochondrial membranes that, through changes in phospholipid fatty acid composition, have a greater rate of futile proton cycling.

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Comp. Biochem. Physiol. B
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4.6

ENDOTHERMIC METABOLISM: FROM CELL TO ORGANISM
A. J. Hulbert, Biological Sci., Univ. Wollongong, 2522 Australia

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 rat mitochondrial phospholipids is greater than across those made from lizard phospholipids. This greater proton permeability is correlated with a more polyunsaturated bilayer. Its 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.

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CALCIUM REGULATION: MECHANISMS AND CONTROL I. CALCIUM REGULATION IN CRUSTACEANS

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 organ level and the regulatory and signalling functions at the cellular level.

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5.2

AN OVERVIEW OF CALCIUM BALANCE IN CRUSTACEANS. Michele G. Wheatly. 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/tissues. 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 uM. There is ongoing debate as to whether skeletal $CaCO_3$ is mobilized to compensate for severe systemic acidosis occasioned by exercise, hypercapnia, acid/aerial exposure etc. In terrestrial settings, where access to external HCO_3^- is limited, EC Ca and HCO_3^- often rise during acidosis but exoskeletal origin has not been definitively proven. Ca dynamics change greatly during the molting cycle. $CaCO_3$ is reabsorbed from the old cuticle which is shed; the new cuticle is mineralized partially with stored Ca but also with de nouveau sources. Postmolt calcification in aquatic species may involve passive mechanisms in SW and active uptake processes that have been extensively reviewed in FW species (2). Postmolt unidirectional Ca uptake appears to be attributed to Ca ATPase and/or a Ca/2H exchanger, and is linked to HCO_3^- and possibly Na uptake. Since the chemical reactions involved in mineralization are pH dependent, the process will only occur in a relatively alkalotic microenvironment and will be impaired during external acidification. Terrestrial species (3) extensively recycle Ca by storing it between molts and reingesting the shed remains; additional Ca is obtained from food and external water. While ecdysis is precipitated by the steroid hormone ecdysone, a direct link between ecdysone and Ca balance has not been established. (Supported by NSF 89-16412)

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5.3

REGULATION OF MINERALIZATION IN CRUSTACEANS. Richard M. Dillaman. Ctr Marine Sci, Univ North Carolina, Wilmington, 28403.

Crustaceans form an unmineralized cuticle below their old one prior to each molt. A mechanism must exist to prevent mineralization of the pre-exuvial cutile prior to the mole and then to rapidly calcify it after the molt. The pre-exuvial cuticle consists of the epi- and exocuticular 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 surfact of the cuticle, and later forms distinct prisms in the exocuticle. Giraud-Guille[1] 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.[2] 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[3]. 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.

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5.5

TRANSEPIDERMAL 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 moult, the new exoskeleton must be calcified and the water calcium salts are moved into the haemolmph 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. Transepidermal 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 or 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 transepithelial 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 $^{45}Ca^{2+}$ uptake and 2) Ca^{2+} sensitive H^+ flux monitored with acridine orange.

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6.1

A 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 (AII) 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 ascribed to the insertion of vesicles that contain 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.[1] 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 given AII show an increase in the expression of WR behavior. Thus, AII could serve to coordinate the physiological and behavioral mechanisms that optimize an animals' ability to rehydrate.[2] 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.[3] Also, toads avoid hypertonic KCl solutions by an amiloride-insensitive mechanism. Recordings from afferent neurons from the ventral skin show a prolonged integrated neural response when the skin is bathed with hyperosmotic 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.
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6.2

REPTILIAN AND AMPHIBIAN 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 endogenous antidiuretic 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 neurohypophyseal hormone, has been suggested to be a diuretic factor in amphibians. More recent evidence in anuran amphibians suggests that adrenergic effectors may also play important roles in the regulation of the kidney, 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 antidiuresis at higher concentrations. Vasoactive intestinal peptide (VIP) causes a glomerular diuresis in preconstricted isolated kidney preparations and is hypotensive 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 potentiated by 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 DCB 9018611 and IBN 9318753.

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6.3

CELL AND MOLECULAR BIOLOGY OF WATER CHANNELS IN AMPHIBIAN BLADDERS AND MAMMALIAN KIDNEY.
A.N. van Hoek and A.S. Verkman. Dept Med & Physiol, UCSF, San Francisco, CA 94143-0521.

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-transporting epithelia and endothelia. A tetrameric assembly of CHIP28 in the lipid bilayer (1) with 4 functional monomers has been established, while topology studies, hydropathy and amphipathy analyses of monomeric CHIP28 indicated 4 to 8 membrane spanning domains. In addition, secondary structure analysis suggested mixed α-helix/β-sheet motifs (2). By homology cloning, several other proteins were obtained, including WCH-CD, a water channel of kidney collecting duct. MIWC, a mercurial-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 CHIP28-like water channel from frog urinary bladder with ~80% identify to human and rat CHIP28 is indicative of functional similarities 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-CD, 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. Carey and Sandra L. Martin. Dept. of Comparative Biosciences, Univ. of Wisconsin, Madison and Dept. of Cell and Structural Biology, Univ. of Colorado School of Medicine, Denver.

Physiological adaptation on proximate or evolutionary time scales ultimately 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 post-translational mechanisms. For example, during the dietary switch from suckling to ruminant modes in sheep, which involves dramatic changes in luminal sugar concentrations, the abundance and activity of the Na^+/glucose transporter is controlled primarily by translational or post-translational events (1). More stable, long-term changes in physiology may require less plastic mechanisms to increase protein activity, such as increased transcription of the corresponding mRNAs. This appears to be the case for certain proteins that are upregulated just before and during hibernation by increasing their mRNA levels (2). 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, evolution of the lysozyme gene family involved gene duplications and divergences to new functions (3). The development of lysozymes that can function at the low pH of gastric contents enabled foregut fermenters 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., post-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 Calif., San Diego, CA 92093-0322.

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 neuromodulatory 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 Calif., San Diego, CA 92093-0623

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 Göttingen 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 Po_2, is related in the following order: counter-current > cross-current> co-current. However, actual performance, in terms of arterial Po_2 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 mass transport models, 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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7.4

CONFLICT AND COMPROMISE IN PHYSIOLOGICAL HOMEOSTASIS.
D.C. Jackson, Dept. of Physiology, Brown University, Providence, RI 02912

The concept of the constancy of the internal milieu and the principle of homeostasis together consititute 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 intrinsic and extrinsic 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 equable environment may be no more "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 regulated variables of 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 C are 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 MONDAY

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 retracing 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 scombroid fishes offers opportunities to identify the molecular and biochemical mechanisms underlying endothermy and also to assess the organismal performance benefits of endothermy. Establishing an evolutionary framework for these analyses of scombroid fishes requires resolution of phylogenetic relationships among the species. We have done this by constructing molecular phylogenies for scombroid 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 tunas. Among cranial endotherms we are examining the evolutionary transition of muscle from a contractile tissue to a thermogenic tissue. In tunas we are establishing the coupling between mode 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., V6T 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 (bimodal 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 O_2 to 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 mass specific 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 provide the strongest data to date that argue for the latter case.

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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 ventilation; whereas the less well known air-breathing fishes, amphibians and reptiles often breathe discontinuously (Ballintijn, 1987). In fishes the respiratory muscles are all inserted around the orobranchial cavity and are 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 extend 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 neuranatomically 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,m 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.

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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 08903-0019

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 S-like K^+ channel similar to that responsible for heterosynaptic modulation of neurotransmitter release in the marine mollusc *Aplysia* (ref #3). In vertebrate respiratory neurons, this channel is activated by γ-aminobutyric acid acting at its β 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 and phrenic motoneurons 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 cardiopulmonary 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 inefficiency, integrated over time, is considerable. Moreover, serious respiratory muscle fatigue must be avoided to prevent insufficiencies, 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 neurons are the kernel for generation of respiratory rhythm
- Respiratory rhythm is generated by a hybrid pacemaker network
- Glutamate is the primary fast neurotransmitter in this network
- Many neurotransmitters modulate respiratory pattern by both pre- and postsynaptic actions
- Several key transmitters affect respiratory pattern by modulating the conductance of various potassium channels

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15.5

Chemoreception and Rhythm Generation in Lower Vertebrates

Remmers, J.E., Kawasaki, H., Kimura, N., Kogo, N., Perry, S.F. Depts. 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 chemoreception and rhythmogenesis. To this end, we have developed and validated a fictively breathing *in vitro* preparation of the brainstem of larval (*R. catesbiani*) and adult (*R. catesbiani and R. pipiens*) frogs. These preparations exhibit rhythmic, alternating, coordinated motor outputs from cranial nerve (CN) and spinal nerve (SN) roots innervating gill (CN VII and VIII), oropharyngeal (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 between cranial nerves V and IX responded to changes in superfusate pH. Fictive lung ventilation in the adult was arrested by the non-NMDA and $GABA_A$ blockers, CNQX bicuculline, and by opioid agonists. Rhythmic bursting persisted after glycinergic 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. $GABA_A$ neurotransmission is essential in the adult but not in the larva. (Supported by MRC grant #MA9719)

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15.6

Brain and Breathing: Snail sets the pace

Naweed I. Syed, Departments of Anatomy and Physiology, Respiratory Research Group, Faculty of Medicine, The University of Calgary, 3330-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 medulla. Our strategy for studying respiratory rhythmogenesis is to develop an invertebrate model system wherein the respiratory behavior is relatively simple and the underlying respiratory network is identifiable and amenable for neurophysiological analysis. In our studies, we use freshwater mollusk, *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 the respiratory behavior in these animals, and identified respiratory motor neurons and interneurons that comprise the Central Pattern Generator (CPG) *in vivo*. To demonstrate that this circuit is indeed sufficient and necessary for the rhythmicity underlying respiration, we reconstructed the network in culture. The *in vitro* reconstructed circuit generated respiratory rhythm similar to that observed *in vivo*. To demonstrate further the significance of respiratory interneurons within the CPG, we first excised a specific respiratory interneuron form the intact animal and showed a fatal deficit in the breathing behavior. We were however, successful in restoring breathing by transplanting the same cell from another snail. This restoration of respiratory behavior coupled with the survival of the animal was due to the integration of the transplanted cells into the host's breathing circuitry. We believe that our snail model provides us with an unparalleled opportunity to explore the mechanisms underlying neural control of breathing at a resolution unapproachable in most vertebrate preparations.
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16.1

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

R. MOTAIS, F. BORGESE, H. GUIZOUARN and F. GARCIA-ROMEU
Laboratoire Jean Maetz, Département de Biologie Cellulaire et Moléculaire du C.E.A., BP. 68, 06230 Villefranche-sur-Mer, France

The Na^+/H^+ exchanger found in the membrane of a nucleated erythrocyte (trout red cell) is an interesting isoform of the human antiporter NHE1: 1) it does not regulate intracellular pH 2) it is activated by ß adrenergic agonists whereas the other isoforms are either insensitive or inhibited by cAMP 3) its explosive activation is rapidly followed by its desensitization 4) its activity is controlled by molecular oxygen, a property related to its physiological function.
This isoform, called ßNHE exhibits a high degree of homology with the NHE 1 antiporter transmembrane domain while the cytoplasmic domain is more divergent . This antiporter is able to restore the functional feature of the trout red cell antiporter (activation by cAMP and PKC activators) when expressed in antiporter-deficient hamster fibroblasts[1]. An examination of the sequence of the cytoplasmic regulatory domain of ßNHE reveals two very close consensus sites for PKA which are not present in the human NHE1. To get insight into the role of these PKA sites and other sites of the cytoplasmic domain, a set of punctual mutagenesis and deletion mutants has been generated[2]. These mutant forms expressed in antiporter-deficient fibroblasts reveal that 1) activation by catecholamines needs serine of the PKA sites. 2) a deletion of the cytoplasmic domain which contains PKA consensus sites abolishes the cAMP activation but does not impair the kinase-C mediated activation. 3) a chimer "NHE1 transmembrane domain/ßNHE cytoplasmic domain" is fully activated by cAMP. These results emphasize the notion that the cytoplasmic domain of the antiporters, although not essential for ion catalysis, is crucial to mediate the various hormonal response . Moreover, at evidence the different signaling pathways do not necessarily converge on "integrator" kinases such as MAP kinase as previously suggested for NHE1 activation.
The kinetics results obtained in presence of phosphatase inhibitors lead us to propose a model for activation and desensitization of ßNHE. It seems likely that regulation of the antiporter involves a recycling mechanim[3].

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16.2

K FLUX PATHWAYS IN TROUT RED CELLS: REGULATION BY OXYGENATION, CELL VOLUME AND PROTEIN PHOSPHORYLATION. A.R. Cossins and Y. Weaver
Department of Environmental and Evolutionary Biology, University of Liverpool, Liverpool L69 3BX, UK.

Trout red cells possess two powerful K flux pathways, the KCl 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 KCl efflux and cell shrinkage, respectively (1,2). The protein phosphatase inhibitors, calyculin A and okadaic acid, inhibit the oxygenation-activated cotransporter but not the hypotonically-activated Cl-independent pathway indicating a controlling role of dephosphorylation in KCl cotransporter activation. A series of protein kinase inhibitors has been screened for effects on these pathways. NEM (*N*-ethyl maleimide) caused the slow activation of the KCl cotranporter and the subsequent addition of calyculin A 'clamped' activity at a fixed level. This clamped activity was volume-dependent indicating that the volume sensor was not part of the serine/threonine phosphorylation system. However, the volume-dependence of the clamped flux was inhibited by other kinase inhibitors. Staurosporine also activated the cotransporter but subsequent addition of calyculin A caused complete inhibition of this flux. Chelerythrine has no effect on the KCl cotransporter but did activate the Cl-independent pathway. These effects are consistent with a model in which cotransporter is activated by a serine/threonine phosphatase and deactivated by a NEM-sensitive kinase. We suggest that staurosporine acts on a separate kinase which controls the activity of the calyculin A-sensitive phosphatase. This indicates that a complex cascade regulates K flux pathways (3).

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16.4

Volume-Sensitive Organic Osmolyte Transport Through a 'Cl'-Channel.
J. Clive Ellory, Jo-Ann Lancaster and Uri 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 hepatocytes 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 Cl-channels there is a certain permeability to monovalent cations. Transport is inhibited by large anionic molecules, including "classical" Cl-channel blockers (NPPB), KCl co-transport inhibitors (DIOA) and Band 3 inhibitors (niflumate, DIDS). Enhanced efflux only occurs when volume changes exceed 25%, i.e. there is a distinct threshold of activation. Raising $[Ca]_i$ 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 in gills by producing a leftward shift of the oxygen equilibrium curve. This is acheived via a reduction of cellular NTP concentration, an increase in intracellular pH or a dilution of haemoglobin within the cell. In teleost fish the first two mechanisms predominate, whereas in lampreys, the haemoglobins of which are insensitive to organic phosphates, the latter two mechanisms are important. In hypoxia-exposed teleost fish the erythrocyte pH is rapidly increased by adrenergic activation of the sodium/proton exchange. At the onset of hypoxia catecholamines are liberated into the blood stream, and the number of functional ß-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 rates of net sodium influx and potassium, chloride (and taurine) efflux. The potassium efflux pathways, activated by cell swelling, are also oxygenation-sensitive. Although catecholamines cause a reduction in cellular ATP concentration, they do not cause the hypoxia-induced reduction of NTP levels. As yet, the pathways regulating cellular NTP concentrations during hypoxia acclimation are not known.

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16.6

THE INTERACTIVE EFFECTS OF STRESS, ADRENOCEPTORS 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_1-adrenoceptors 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 β_1 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, reduces the number of cell surface β_1 receptors (down-regulation). During actual repeated stress (e.g. daily handling), cortisol and catecholamine levels are both elevated and presumably they 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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ANHYDROBIOSIS

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 80262

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 steps. 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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17.2

MODELS FOR ANHYDROBIOSIS: STUDIES ON CONFORMATIONAL STATES OF DRY PROTEINS. Steven Prestrelski. 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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17.3

MODELS FOR ANHYDROBIOSIS: PRESERVATION OF LIPID BILAYERS DURING DRYING. Lois M. Crowe and John H. Crowe. 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 lyotrophic 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 liquid crystalline state while dry, is necessary for bilayer preservation. More recent experiments have demonstrated that it is necessary that the phosopholipid: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.

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17.4

MODELS FOR ANHYDROBIOSIS: STABILIZATION OF BIOLOGICAL MEMBRANES DURING DRYING. John H. Crowe, Samuel B. Leslie, and Lois M. Crowe. 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 IBN-9308581 from the National Science Foundation and N00179 from the Office of Naval Research.

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17.5

Studies on Intact Anhydrobiotes: Lipid Phase Transitions and Long Term Stability. Folkert A. Hoekstra, Dept. of Plant Physiology, Wageningen Agricultural University, Wageningen, The Netherlands

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 imbibition. Employing FTIR, shifts of the symmetric CH_2 vibration band were noticed in intact pollen in relation to its moisture content. On account of such behavior we assigned the CH_2 absorption band around 2850 cm^{-1} to phospholipids in the membranes (1). The calculated T_m ranged from -6°C in hydrated pollen to 32°C in very dry pollen. Dried isolated membranes had a T_m of 60°C, which was reduced to 30°C in the presence of sucrose, the major soluble carbohydrate in the pollen (2). We conclude that sucrose effectively reduces the rise of T_m of membrane phospholipids *in situ* with desiccation. Thus, it may provide desiccation tolerance. Certain seeds tested also showed reduced rise of T_m with drying. Aging leads to imbibitional leakage of solutes. This may be explained by the observed gradual rise of T_m 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 carbohydrate species involved will be discussed.

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17.6

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

Pedro Soares de Araujo[1] and Anita D. Panek[2]

[1]Depto. de Bioquímica, Instituto de Química, Bl. 10T, Universidade de São Paulo, São Paulo 05508-900, Brazil [2]Depto. de Bioquímica, Instituto de Química, CT, Bl. 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. In isolated plasma membrane vesicles trehalose transport is vectorial with the pH gradient thus providing a means for the presence of trehalose on both sides of membranes under stress conditions. Genetic evidence indicates that the expression of this transporter is regulated by the same gene that regulates maltose metabolism in *Saccharomyces*. The trehalose permease gene seems to correspond to the gene that was formerly described as one of the cryptic genes of the MAL system, widely distributed among yeast strains.
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FROM MYXINE TO MAN: THE PHYSIOLOGY OF BLOOD VOLUME REGULATION

18.1

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 H_2O excretion. This presentation introduces studies carried out to determine the role of physical factors (renal arterial hydrostatic pressure (RAP) and plasma colloid osmotic pressure (COP)) in the short and long-term control of BV. COP: Expansion of the extracellular space with an iv isotonic NaCl with neural-hormonal controllers and RAP held constant or inactivated is associated with excretion of more than 90% of the volume load over 1-2 hours. This is accomplished by renal responses dependent on the dilution of plasma protein and the reduction of COP. Equivalent isooncotic BV expansion under similar conditions fails to increase Na and H_2O excretion. The mechanism for the reduced tubular Na reabsorption is related to an increase of renal papillary blood flow, washout of the medullary urea gradient, and increased renal interstitial fluid pressure. COP remains unchanged with chronic increases of salt intake and is not responsible for the sustained increase of Na and H_2O excretion. Pressure-diuresis: If RAP rises with volume expansion, pressure-diuresis contributes importantly to the normalization of BV (acutely and chronically) with a 2X rise of urine flow rate for each 10 mmHg rise of pressure. With a chronic increase of daily salt intake, neural and hormonal systems are normally activated and participate importantly in renal excretion and volume regulation. 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 SALTWATER HAGFISH AND ELASMOBRANCHS. Siribhinya Benyajati and Stanley D. Yokota. Depts. of Physiology, Univ. Oklahoma HSC, Oklahoma City, OK 73190 and West Virginia Univ., Morgantown, WV 26506

Both hagfish and elasmobranchs maintain their body fluid osmolality slightly hyperosmotic to the marine environment, therefore they both are subjected to net osmotic water influx. The extracellular fluid volume (ECFV) of hagfish is twice that of elasmobranchs (250 vs. 120 ml/kg). The blood volume in both groups is a higher proportion of the ECFV than in other vertebrates. Limited evidence suggests that hagfish possess some ability to regulate their ECFV, probably through alterations of the filtration rates of their kidneys. Since there is no net fluid reabsorption in the hagfish kidney, the urine output equals the filtration rate. Although the nature of the mediator(s) of the volume regulation is not known, both catecholamines and atrial natriuretic peptides (ANP) occur naturally and are vasoactive in hagfish. Elasmobranchs also face net Na^+ influx; the excess NaCl is excreted through rectal salt glands via Na-K-2Cl cotransport. Salt secretion by the gland is modulated by a variety of neurotransmitters and hormones (eg. VIP, rectin, adenosine, ANP). The kidney is another organ for volume regulation in elasmobranchs; sharks alter their rates of glomerular filtration (GFR) to regulate water excretion in response to changes in salinity. Elasmobranch renal function is modulated by plasma catecholamine levels which change with the volume status of the animal. Various peptide hormones (ANP, VIP, prolactin, and arginine vasotocin) appear to play important roles in modulating elasmobranch GFR to meet volume regulatory demands. The role of the renin-angiotensin system in elasmobranchs remains equivocal but exogenous angiotensin II displays catecholamine-mediated vasoactivity. (Supported by L.P. Markey Charitable Trust and NSF)

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18.3

VASCULAR COMPARTMENT AND VOLUME REGULATION IN TELEOSTS. K.R. Olson. Ind. Univ. Sch. Med., South Bend Ctr., U. Notre Dame, Notre Dame, IN 46556.

Bony fish thrive in both hydrating, salt depleting (freshwater) and dehydrating, salt-loading (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 hypo- and hyper-osmotic 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 ml·kg^{-1}) are consistently lower than those reported for other vertebrates, while labeled albumin produces considerably higher values (83 ml·kg^{-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-inaccessible, vascular compartment. This secondary system, apparently unique among vertebrates, arises from primary systemic arteries in the form of a myriad of small arterioles that anastomose to perfuse gills, skin and fins. Secondary circulation volume has been estimated to be 50% larger than the primary with a circulation time in excess of eight hours. Estimates of extracellular volume in teleosts usually fall within 180-250 ml·kg^{-1}. Capillary hydraulic pressure and effective plasma oncotic pressure are not known but both may be below 10 mmHg. The reported absence of a lymphatic system in teleosts complicates the issue of transcapillary fluid balance. Factors such as hemorrhage or atrial natriuretic peptide stimulate translocation of interstitial fluid into the vascular compartment and suggest that both intravascular and interstitial fluid compartments may be regulated by physiological stimuli. Supported by NSF Grant No. IBN 9105247.

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18.4

BLOOD VOLUME CONTROL IN AMPHIBIANS. Stanley S. Hillman Biology. Portland State University, Portland, OR 97207-0751

Amphibians are excellent models for the study of blood volume control, since relatively rapid increases and decreases of blood volume are natural biologic stresses in aquatic and aerial environments respectively. Intravenous hypervolemic challenge with a volume equivalent to 10% of body mass led to a decrease in TPR, a slight decrease in Pa, and a large increase in Pv. There is also evidence for ANF mediated increased capillary hydraulic conductivity with volume expansion. These conditions favor loss of fluid in the kidneys and to the lymphaic space. A new volume equilibrium is established in the first hour which is 50% larger than control indicating an increase in circulatory compliance. Graded hemorrhagic challenge and dehydration lead to mobilization of extravascular fluid to maintain blood flow. Species differ in their ability to compensate for hypovolemia and dehydration, as terrestrial species are still able to maintain maximal blood flow rates after over 50% of their initial blood volume is removed. The source of the fluid used to compensate for hypovolemic stress appears to be lymph moblized via lymphatic hearts and not transcapillary oncotic uptake. Upon depletion of lymphatic reserves, TPR increases in an attempt to stablize Pa. This increase in TPR appears to be neurally rather than hormonally mediated. Bladder water and cutaneous uptake can be utilized to stabilize blood volume when reserves or free water are available. Mobilization is principally mediated by hormonally induced permeability changes leading to enhanced osmotic influx. Contributions of nephrostomes (peritoneal funnels) are of minor import when compared to lymph and bladder water fluxes, though little is known concerning interspecific differences in number and control. There are conflicting data on the relative permeability of amphibian circulatory systems.

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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 extavascular fluids (1). Such volume compensation appears to be attributable to strong reflex vasoconstriction in peripheral 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 interstitium occurs in response to exercise, elevated blood pressure, and gravitational pooling of blood during upright posture. In spite of the lability 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
Yoshio 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 severer 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 volume receptors to monitor extracellular fluid volume are located in the extravascular, 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 dipsogenic 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 ALD 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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CALCIUM REGULATION: MECHANISMS AND CONTROL II. CALCIUM REGULATION IN LOWER VERTEBRATES

19.1

CALCIUM REGULATION IN AQUATIC VERSUS TERRESTRIAL POIKILOTHERMIC VERTEBRATES. James C. Fenwick. Dept. Biology, Univ. Ottawa, Ottawa, Ontario, Canada, K1N 6N5

Because of the myriad biochemical and physiological effects of calcium 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 hypercalcaemia 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 bony fish is dominated by the antihypercalcemic hormone, stanniocalcin, which operates primarily by reducing calcium uptake through the gills. Wholly aquatic amphibians appear to be uniquely sensitive to calcitonin, another hypocalcemic hormone. Conversely, calcium regulation in primarily terrestrial amphibians, and the reptiles, follows the avian and mammalian model and is under the primary control of the hypercalcaemic 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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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 movements 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 areas. 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).

Transcellular 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.

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19.3

CELLULAR CALCIUM TRANSPORT IN FISH: UNIQUE AND UNIVERSAL MECHANISMS.
G. Flik and S.E. Wendelaar Bonga. Dept. Animal Physiol., Fac. Sci., Univ. Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.

Fish take up Ca^{2+} via gills and intestine and excrete Ca^{2+} via kidneys and intestine. Branchial ionocytes, enterocytes and nephric cells are specialized for transcellular transport of Ca^{2+}. Entry of Ca^{2+} over the apical membrane, the rate limiting step, is regulated by stanniocalcin and, at least in enterocyte brush border membrane vesicles, carrier-mediated. Extrusion of Ca^{2+} from the cell to the blood is driven by a Ca^{2+}-ATPase (kidney), a Na^+/Ca^{2+}-exchanger (intestine) or both (gills). In tilapia (*Oreochromis mossambicus*) kept in fresh water (FW), prolactin enhances Ca^{2+} uptake and concurrently controls the density of Ca^{2+}-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^{2+} transport at the apical membrane. In SW tilapia, as compared to FW tilapia, epithelial Ca^{2+} influx is comparable in gills, but lower in intestine and kidneys. Accordingly, Ca^{2+}-ATPase and Na^+/Ca^{2+}-exchange activities in FW and SW gills are similar. The extrusion of calcium from the enterocyte is dominated by a Na^+/Ca^{2+}-exchanger rather than by a Ca^{2+} ATPase, in line with the dependence of intestinal Ca^{2+} uptake on the Na^+ status of the epithelium. Seawater tilapia drink significantly but absorption of Ca^{2+} via the intestine is minimized; in parallel, Na^+/Ca^{2+}-exchange activity is decreased. Renal cells of seawater fish contain less Ca^{2+}-ATPase, in line with decreased needs for Ca^{2+} reabsorption. Thus, stanniocalcin, unique for fishes among the vertebrates, exerts a universal action in fish Ca^{2+} transporting epithelia. Extrusion of Ca^{2+} from fish Ca^{2+} transporting cells depends on ATP- and Na^+-gradient driven Ca^{2+}-pumps universal among vertebrates, but unique in their tissue distribution in fish.

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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^{2+} from environmental water and the small intestine which takes calcium from food. The kidneys and bladder limit execretion. Bone, endolymphatic sacs and layers in the skin of some species shuttle Ca^{2+} to and from extracellular fluid. Early attempts to characterize Ca^{2+} 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^{2+} solutions they take up Ca^{2+} against an electrochemical gradient in a manner that is dependent on external (Ca^{2+}) and saturable. Influx and net uptake are enhanced when frogs are acclimated to distilled water and are stimulated by parathyroid hormone. Similar Ca^{2+} transport exists in the skin of Xenopus laevis and Ambystoma tigrinum.

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19.5

CALCIUM METABOLISM IN EMBRYONIC REPTILES AND BIRDS.
Mary J. Packard and *Nancy B. Clark. Colorado St. Univ., Ft. Collins, CO 80524.

Embryos of oviparous reptiles and birds must maintain calcium homeostasis while mobilizing large quantities of calcium from the yolk and eggshell. The same calcium-regulating hormones that control calcium status of adults are assumed to control calcium status of embryos, but the target organs for calcium regulation may be different in embryos and adults. The yolk sac, which mediates transfer of yolk calcium, and chorioallantois, which mediates the release and transport of shell calcium, are potential targets for calcium-regulating hormones during embryogenesis. The presence of receptors for the vitamin D hormone (calcitriol) in both epithelia of chicken eggs, the extreme calcium-deficiency of vitamin D-deficient embryos, and the consistent hypercalcemia elicited by exogenous hormone indicate that calcitriol plays an important role in regulating calcium metabolism during embryogenesis. The roles of parathyroid hormone and calcitonin in embryonic birds are less clear, and the potential for hormonal control of calcium homeostasis in embryonic reptiles has not been examined. [supported in part by NSF (IBN-8718191 & IBN-9407136).]

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19.6

HORMONAL CONTROL OF CALCIUM REGULATION IN VERTEBRATES
Pang, P.K.T., Shan, J. and Chiu, K.M. Department of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

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 some special developmental periods such as growth and reproduction. However, the more important use of calcium is the continuous control of activities 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 $(OH)_2$ 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.

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EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: COMPARATIVE ASPECTS

20.1

EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: INVERTEBRATES.
M.J. O'Donnell. Biol, McMaster Univ, Hamilton, Canada.

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 added and salts are removed (1). In ocypodid crabs, by contrast, the antennal gland acidifies urine (pH = 5.5) and elevates ammonia concentrations (> 100 mM; ref. 2). Branchial exchange of sodium/ammonium and chloride/bicarbonate drive gaseous ammonia release from the branchial chamber of a geograpsid land crab. Gaseous ammonia release by terrestrial isopods involves rapid mobilization of ammonia into the hemolymph from a sequestered form, followed by excretion into the thin film of fluid on the ventral pleopods, and volatilization. In high humidity, colligative lowering of water vapor pressure in pleon fluid by > 4M NaCl couples ammonia release to net gain of atmospheric water. Between bouts of ammonia release, ammonia is sequestered in non-toxic form, primarily as glutamine and glutamate (3). Nonetheless, isopods and some other crustaceans are tolerant of extraordinarily high hemolymph ammonia levels. In desert locusts, transport by the ileum produces ammonia concentrations as high as 400 mM and ammonia is excreted at 3 times the rate of total urate (Phillips et al., Physiol. Zool. 67, 95-119).

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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)[1]. 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[2], and some are facultatively ureotelic, e.g., selected toadfishes[3]. A fully functional hepatic ornithine-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 synthetase (GNS) activity, which traps ammonia nitrogen, shunting it towards urea. Several laboratory treatments can induce this switch (e.g., air-exposure, NH_4Cl 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/head 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).

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20.3

EXCRETION OF NITROGEN-CONTAINING COMPOUNDS: AMPHIBIANS. Vaughan H. Shoemaker. Univ. Calif., Riverside, CA. 92521

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 hyperosmotic 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 urea concentrations of 150 mM and U/P ratios of urea 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. A few amphibians can remain active and feed when deprived of water for long periods. These produce uric acid as the primary nitrogen waste and require little water for nitrogen excretion beyond that contained in the food. Aestivating amphibians and amphibians living in hypersaline environments produce and store urea. This can be beneficial in reducing water potential of body fluids. Renal mechanisms of urea excretion are diverse and worthy of further study.

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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 NH_4^+. 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 flow-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 ophidian reptiles. Reabsorption may involve high affinity electrogenic Na^+-Cl^--taurine cotransport at luminal membrane and electroneutral 2-3 Na^+-1Cl^--1taurine cotransport at basolateral membrane, but secretion steps are not defined.

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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 concentration, 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. 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, as part of the urine are also moved into the lower GI tract. Within the colon and ceca are large populations of bacteria. A segment of the bacterial population is specialized to degrade uric acid. Some of the large quantity of protein present in the urine may also be degraded in this area of the GI tract. For uric acid, evidence suggests that some of the nitrogen may be recycled, 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 B. 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 the 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[1,2]. 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.

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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 extraocular muscle specialized for heat production[1]. Tunas warm the cranial compartment, the viscera, and the swimming muscles in a strategy referred to as systemic endothermy[2].

Examining 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)[3] 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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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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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 analysing 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 phylogenetic trees, develops estimates of the rates of change of the discrete characters, and tests the hypothesis of correlated evolution without relying upon reconstructions of the ancestral character states. A likelihood ratio test statistic is used to discriminate between two models that are fitted to the data: one allowing only for independent evolution of the two characters, the other allowing for correlated evolution.

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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 O_2 consumption, heart rate, hematocrit, % muscle fiber types, relative organ masses, in vitro 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). Often the focus is to elucidate physiological mechanisms (e.g., does relative limb length predict interspecific differences in locomotor performance?) or adaptive significance (e.g., do species inhabiting high altitudes have enhanced cardiovascular/pulmonary function?). Conventional statistical methods are invalid for analyzing comparative data, however, because they assume that mean values for different species represent statistically independent pieces of information. This assumption is violated because species are hierarchically related. Thus, methods have been developed that use 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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Outline of the simulated null distribution approach to testing hypotheses about comparative data and application to analysis of differences in home range area between mammalian carnivores and herbivores, yielding surprising results; discussion and references on treating polytomies.

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 O_2 flows through 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 built 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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A series of eight papers testing the principle of symmorphosis at each step in the oxygen pathway. Maximal O_2 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
Experientia
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 lactation.

MORNING PLENARY LECTURE TUESDAY

30.0

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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31.1

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

Despite tremendous diversity in respiratory structures, mechanics and medium, 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 hypercapnia appear to be mediated exclusively by their peripheral (branchial) chemoreceptors. Removal of chemo- and mechanoreceptor 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 chemo- or mechanoreceptor stimulation. 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 chemoreceptor feedback, but a variety of single breath and periodic breathing patterns can be produced by altering steady state levels of central and peripheral stimulation. Mechanoreceptors mediate lung inflation and deflation reflexes, and may terminate bouts of breathing. Air flow control within bouts is not understood in buccal pump breathers.

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DEVELOPMENT OF NEURAL SYSTEMS FOR BIMODAL RESPIRATION. A.I. Pack, L. Kubin, R.J. Galante, G-S. Liao, A.P. Fishman. Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, Pennsylvania

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 amphibia 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 amphibia (*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 rhythmicity 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 rhythmicity 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

DEVELOPMENTAL TRANSITIONS. Sandra J. England. Dept. of Pediatrics, UMDNJ-Robert Wood Johnson Med. Sch., New Brunswick, NJ 08903.

The respiratory control system is functional in the mammalian fetus, capable of generating rhythmic diaphragmatic contraction and responding to peripheral mechano- and chemoreceptor 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 mechano- and chemoreceptors 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 maturational 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, the 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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31.4

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 (temporary) alteration in synaptic strength or cellular properties, adjusting or even transforming network operation. Monoamines (*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 HL36780).

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31.5

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 HL44889 and HL50374.)

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ENVIRONMENTAL AND PHYSICAL DETERMINANTS OF MUSCLE PERFORMANCE CAPACITIES

32.1

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 (T_4) 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 T_4, 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 (7 weeks old), the development of all the variables measured are the same in both populations. T_4 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 metabolite 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 therefore means masking of catalytic potentials; metabolic activation means unmasking latent catalytic potentials. At this time, only one metabolite 'signal' - oxygen delivery - correlates directly with (upwards or downwards) change in ATP turnover rate and since oxygen regulatory effects clearly occur at concentrations well above the apparent Km for mitochondrial metabolism, it is postulated that an oxygen sensing system must be 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 multipe enzymes in pathways of ATP turnover) must be satisified for this kind of control to be workable.

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32.3

IS GROWTH RATE A SIGNIFICANT MODULATOR OF MUSCLE METABOLIC CAPACITIES? Helga Guderley[1], Dany Pelletier[1,2] and Jean Denis Dutil[2]? [1] Dép. de Biologie, Univ. Laval, Québec, Canada; [2] Institut Maurice Lamontagne, Fisheries and Oceans, Mont Joli, Québec, Canada

In contrast to the well-known effects of starvation, the impact of growth rate on muscle metabolic capacities has only recently come under study. 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 cod with those of wild cod suggest 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.

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32.4

Thermally Induced Changes in Fish Oxidative Muscle: The Interplay of Structure and Metabolic Poise. B.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 > 1.8-fold between 25° and 5°C (1). This highly viscous cellular milieu at cold temperatures may impede diffusional movement of 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 metabollism of their skeletal muscle fibers. The fraction of oxidative fiber volume occupied by mitochondria increases from 0.28 ± 0.02 to 0.45 ± 0.02 during acclimation of striped bass from 25° to 5°C and typically ranges from 0.35 - 0.5 in fibers from antarctic polar species (2). Both cold acclimated temperature zone species and polar fish species display preferential reliance upon oxidation of fatty fuels to support aerobic energy metabolism (2,3). Proliferation 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_{cat}. Expansion of the mitochondrial cytoplasmic and mitochondrial compartments, compensating for reductions in molecular diffusion coefficients at cold temperature. High mitochondrial volume densities characteristics of cold adapted animals may result in maintenance of a high energetic status of the cellular adenylate pool, restricting glycolytic flux and favoring preferential oxidation of non-carbohydrate substrates.

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32.5

Temperature and locomotion in fish: crossbridges to whole animals

I.A. Johnston, Univ St Andrews, Scotland

Short-horned sculpin (*Myoxocephalus scorpius* L) around the coast of Scotland experience average temperatures of around 5°C in winter and 15°C in summer. "Fast-starts" used for prey capture have been shown to be modified following several weeks thermal acclimation. Parameters which can be altered include 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 (P-V) relationship of live muscle fibres. The P-V relationship at 5°C is significantly less curved in muscle fibres from 5°C- than 15°C-acclimated fish. After normalising the curves for P_o and V_{max} 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-aclimated 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 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 *in vivo* muscle work and the molecular mechanisms underlying changes in contractile properties will be discussed.

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ONTOGENY OF CARDIOVASCULAR SYSTEMS I: MECHANISMS

33.1

CARDIOVASCULAR DEVELOPMENT IN AMPHIBIANS. Warren 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 1-2 mg body mass to about 10 mmHg at 1 g and 20-30 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 $mm^3/min^{-1}/mg^{-1}$ at 2 mg body mass to about .7 $mm^3/min^{-1}/mg^{-1}$ at 1 g body mass. Peripheral resistance correspondingly decreases sharply during development. While there are some notable quantitative 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 anuran 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 Cantabrai, 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 condense 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 single 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 in the center of the heart. Concomitant with all these changes, cells of extracardiac origin (epicardium, blood vessels, neural crest, nerve fibers) reach the heart and form new systems that become integrated in the developing heart (1-3). While early morphogenesis depend on the expression of specific traits, and shape changes involve deformation of epithelial sheets, late heart organogenesis appears to be governed by mechanisms of differential tissue growth and tissue remodeling. Emphasis is placed on inductive events, biochemical differentiation, cellular and extracellular signals, and the possible role of the different cell activities in the regulation of shape and function of the developing heart.

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33.3

GENETIC DISSECTION OF CARDIOVASCULAR DEVELOPMENT IN THE ZEBRAFISH

M. Fishman. Massachusetts General Hospital, CV 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 cardiovasculature 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.Driever 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.

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33.4

VASCULOGENESIS/ANGIOGENESIS DURING DEVELOPMENT.

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

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 indicate 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 as yet well understood, recent data suggest that its regulation involves both cellular and extracellular events.

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Entire coronary endothelial vasculature originates from an extracardiac source (liver region).

33.6

REGULATION OF VASCULAR DEVELOPMENT. T.H. Adair, J. Hang, and J-P. Montani. Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS 39216

Long-term imbalances 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. It is not yet certain that VEGF released from hypoxic tissues mediates the vessel growth that occurs; however, it is known that exogenous administration of VEGF can stimulate growth in ischemic tissues. The feedback control hypothesis of vessel growth described here may apply to the angiogenesis in skeletal muscle caused by electrical stimulation, endurance training, exposure to a cold environment, as well as the vascularization of the corpus luteum, tumor angiogenesis, vessel growth in wound healing, and even the overall growth of the cardiovascular system in a developing organism. (Supported by HL51971, HL42402, and HL02117).

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34.1

THE KIDNEY AND VERTEBRATE EVOLUTION. Leonard B. Kirschner
Washington State University, Pullman, WA 99164.

Sixty years ago Marshall and Smith (M-S) suggested that the glomerular nephron was suited to excrete a water load, was found in all aquatic vertebrates that faced water loading, but was reduced in marine (SW) teleosts some of which became aglomerular. They concluded that the organ must have developed in fresh water (FW), hence that vertebrates evolved in FW (1). Later, data on invertebrate renal function made it clear (2,3) that kidneys in annelids, molluscs and crustaceans produced a blood ultrafiltrate that was processed as in the vertebrate nephron. Most marine invertebrates are isosmotic with SW and have 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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34.2

Functional Morphology of Renal Epithelia in Fish
Marlies Elger
Institute of Anatomy and Cell Biology, University of Heidelberg, Germany

The morphology of renal epithelia of Agnatha, Elasmobranchiomorphi, and Actinopterygii is reviewed. The study of glomerular and aglomerular fish leads to the concept of two basically different strategies in the vertebrate proximal tubule. Reabsorptive functions, which are associated with morphologically distinct features (e.g. apical endocytotic apparatus), are closely related to the ultrafiltration process of the glomerulus. Both glomerulus and reabsorptive segments establish a functional unit and they 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 lacking in marine fish because of their need to conserve water could be verified only for many teleosts, which represent a rather young side 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 collecting duct in all vertebrate classes, and are missing only in teleosts.

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34.3

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: Mg^{++} 154 ± 28 and SO_4^{--} 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 glomeruli (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 be lacking in lampreys, was pressor and antidiuretic (Broadhead and Rankin, in preparation).

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34.4

GLOMERULAR FUNCTION OF THE *IN SITU* PERFUSED TELEOST KIDNEY. J.Anne Brown and Shehla Amer. Dept. Biol. Sci., Univ. Exeter, Exeter EX4 4PS, UK.

Teleost 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. 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 (NF), and non-arterially-perfused (NP)]. 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^{-11}M), demonstrating a potent glomerular antidiuretic action by reducing the population of filtering glomeruli to approx 30%, while a similar proportion of NP glomeruli emerge, (4) demonstrated that 10^{-11}M endothelin-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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Presents an overview of *in vivo* studies of glomerular function in fish

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 hyperosmolar 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 complicated 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 bundles; 2. a negative gradient of urea is produced by countercurrent multiplication of a hairpin loop of neck segment and proximal tubule segment PIa 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^{-12} and $2x10^{-11}$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 from FW to SW, eels showed a transitory rise in plasma AVT (2). Acute blood volume expansion in SW-adapted flounder reduced plasma AVT 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 V_1-type receptor for AVT it appears that the teleost nephron also possesses a V_2-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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34.7

THE RENIN-ANGIOTENSIN SYSTEM IN SHARKS. Sara M. Galli. Department of Physiology, University of Florida, Gainesville, FL 32610.

Evidence for the presence of an active RAS in two species of sharks, *gynglimostoma cirratum* (Nurse shark) and *squalus acanthias* (dogfish) is presented: Northern blot analysis shows the presence of angiotensin (Ao) mRNA in liver, the generation of increasing amounts of Ang I *in vitro*, and significant rise in Ang II levels in captopril treated sharks. Angiotensin I, II, III and Ang metabolites were identified and quantified in plasma, kidney, brain and pituitary. In nonanesthetized dogfish, increasing concentrations of DF-Ang II (5 to 100 ng/Kg^{-1} b.w.) raised blood pressure in a dose dependent fashion and a significant rise in plasma epinephrine was observed. The blood pressure response to Ang II was blocked by the Ang II type-1 receptor blocker (Losartan), only in Nurse shark but not in dogfish. In this specie hypotension and hypovolemia significantly raised plasma Ang II levels. High concentration of Ang II (ng/g tissue) and high specific binding for ^{125}I-Ang II was found in the Nurse shark rectal gland (RG). This binding was not displaced by AT_1 or AT_2 receptor blockers, suggesting the presence of a unique third type of Ang II receptor in the Nurse shark RG. The Ang II receptor that mediates the blood pressure response in this specie may be similar to AT_1 ANG II receptor subtype.

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34.8

CONTROL OF RENIN RELEASE IN TELEOSTS. Hiroko Nishimura and Zelian Qin. Dept. of Physiology, Univ. of Tennessee, Memphis, TN 38163

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 channels and a subsequent increase in intracellular Ca^{2+} in renin secretory cells appear to inhibit renin secretion (Nishimura and Madey, 1989), whereas a calmodulin antagonist or removal of extracellular Ca^{2+} increases renin release. In contrast, stimulation of β-adrenoceptors or cAMP production does not evoke the renin release. Furthermore, neither changes in interstitial osmolalities nor application of cGMP, prostaglandin E_2, or arachidonic acid increased renin secretion, suggesting that Ca^{2+} mediates an inhibitory message for control of renin release underlying the baroreceptor mechanism. Since renin secretory cells are modified vascular smooth muscle (VSM) cells, we intended to characterize Ca signaling in toadfish VSM. Cytosolic Ca^{2+} signals determined by a fluorescent indicator (fura-2) were increased by K^+, Bay K 8644, and transiently by ANG II. VSM membrane fraction contains specific ^{125}I-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

35.2

ECOLOGICAL CORRELATES OF SWIMMING PERFORMANCE IN ENDANGERED SPECIES OF CYPRINID FISHES FROM THE SOUTHWESTERN U.S. Malcolm S. Gordon, Ana-Esther Escandon and Itai Plaut. UCLA, Los Angeles, Ca. 90024-1606

Many species of freshwater bony fishes native to the southwestern U.S. have suffered serious reductions in populations during the past century. Some are extinct, others are legally designated as endangered or threatened, still others are near designated status. Rational design of recovery and maintenance plans requires, among other things, knowledge of ecologically relevant features of their activity metabolism. We have studied 3 species of cyprinid fishes in these respects: 2 native species (the endangered bonytail chub, Gila elegans, and the increasingly rare arroyo chub, Gila orcutti) and one species widely introduced to the southwest which is an important competitor and potential displacer of several native species (the fathead minnow, Pimephales promelas). The energetics of swimming differ in the 2 native species. The introduced species is closer to the arroyo chub. At water temperatures of 15, 20 and 25C the slopes of linear regression lines for mass-specific aerobic metabolic rates vs relative swimming speeds were 3-8 times higher for bonytail chubs as compared with arroyo chubs. Standard metabolic rates calculated from the intercepts of these lines were lower for bonytail than for arroyo chubs. In nature bonytails live in rapid, oxygenated, cooler streams, arroyo chubs in slower, hypoxic, warmer flows.

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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 and behavior in flow regimes like those near diversions. Depending on acclimation temperature and salinity, delta smelt tolerated temperatures from <7 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 exceed 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 canorus) 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 stressful and 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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35.6

ROLE OF THE REPRODUCTIVE PHYSIOLOGIST IN CONSERVING MAMMALIAN BIO- AND GENETIC DIVERSITY. David E. Wildt, National Zoological Park, Smithsonian Institution, Washington, DC 20008

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 sufficient 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 Genome 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 gene 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 NICHE 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 37°C. The bacteria adapted much 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 decreased; 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

METABOLISM, SWIMMING AND MUSCLE FUNCTION IN ANTARCTIC FISH. C.E. Franklin. St. Andrews, Gatty Marine Lab., St. Andrews, Fife. KY16 8LB, Scotland, UK.

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 metabolic power output largely involves increasing the numbers and cristae density of muscle mitochondria. The maximum rate of oxygen consumption of isolated red muscle mitochondria from the Antarctic fish (*Notothenia corriceps*) 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 urine by reabsorbing filtered solutes without filtered water. Diluting ability is correlated with environmental need to excrete excess water. Significant concentrating ability is limited to mammals and birds and depends on concerted function 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 concentration in arid environments, but renal conservation of water in birds is related particularly to regulation of filtration and to excretion of urate. 3) Regulation of water excretion via urate excretion. Regulation of urate excretion may be related to number of filtering nephrons.

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36.4

Control of salt gland function in marine birds

Gerstberger, Rüdiger, W.G. Kerckhoff-Institute, MPI for Physiol. and Clin. Res., D-61231 Bad Nauheim, F.R.G.

Marine birds possess supraorbital salt glands to eliminate excess NaCl from their extracellular body fluid (ECF). Increases in ECF tonicity and volume represent the physiological stimuli for salt gland secretion (1). Changes in ECF tonicity are monitored by hypothalamic tonicity receptors which have been characterized electrophysiologically. Alterations in ECFV are monitored by systemic volume receptors utilizing angiotensin II (ANGII) as afferent messenger to the brain. *Via* 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 parenchymal tissue and arterioles. As a potent co-transmitter, VIP stimulates salt gland blood flow and secretion through binding to membrane-intrinsic receptors with cAMP as second messenger. Mimicking sympathetic innervation, norepinephrine and alpha2-agonists cause vasoconstriction of the salt gland vasculature with slightly diminished secretion. Neuronally released nitric oxide as non-cholinergic neuromodulator surprisingly reduces both salt gland blood flow and NaCl excretion. With regard to hormonal control, steroid hormones and prolactin appear to fulfill merely permissive functions, and the antidiuretic hormone does not influence salt gland secretion. 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.

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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

Oreochromis alcalicus grahami lives in geothermal pools at the edge of Lake Magadi where pH = 10; $CO_3^{=}$ = 265, Na^+ = 342 and Cl^- = 108 mequiv.l^{-1}; osmolality = 525 mOsm.kg^{-1}; temperature (23 - 42+°C) and P_{O2} (<20 - >400 torr) fluctuate diurnally. The difficulty in excreting ammonia at such alkaline pH has been solved by switching to an alternate N-product, with complete ureotelism through expression of the ornithine-urea cycle (1). Ammonia tolerance is exceptionally high (2). Adaptations for acid-base balance include regulation of remarkably high extra- and intracellular pH's (3). Despite an outwardly directed Cl^- gradient, gill chloride cell morphology is typical of seawater teleosts, suggesting a branchial role in coupled Na^+ and base excretion. Tolerance of low O_2 is aided by natural co-variation of environmental temperature and P_{O2} in combination with a very high Q10 of metabolic rate in the critical range. Additional adaptations include a short blood-water diffusion distance, high blood O_2 affinity, absence of a Bohr effect, and a capacity for supplementary air-breathing (supported by NSERC, NATO, NSF and National Geographic).

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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 (J) 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, inter-species 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 hummingbirds 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 unit mitochondrial volume and per unit cristae surface area lead to intriguing biochemical questions concerning molecular architecture as well as physiological questions about the role played by mitochondria in setting the upper limits to VO_2max.

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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.

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37.3

Testing the hypothesis of symmorphosis: are linked functional capacities designed economically ?

Ewald 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 coadjusted to overall functional capacity. We have tested this hypothesis on the pathway for O_2 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 $V_{O2}max$. In contrast, the lung shows a limited excess capacity for O_2 uptake, but other studies suggest that this may be related (1) to variations in the environmental O_2 and (2) to a limited morphogenetic capacity of this organ. In recently extending these studies to the matched supply of substrates for oxidative metabolism (glucose and fatty acids) we arrived at the conclusion that the muscle microvasculature is adjusted to the needs for O_2 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.

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37.4

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 matching of supply and demand may be an unlikely outcome of evolution. (1) Numerous processes (e.g. routine homeostasis, 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 approximate 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.

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EVENING PLENARY LECTURE

38.0

Juvenile Hormone and Insect Metamorphosis: The Status of Its "Status Quo". Lynn M. Riddiford, Department of Zoology, University of Washington, Seattle, WA 98195.

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 thus continued growth. In the final larval stage ecdysteroids acting in the absence of JH cause a switch in developmental program to that of the pupa; then a similar switch occurs in the pupa at the outset 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 epidermis 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 high affinity, nuclear binding protein for JH (JP29) 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-JP29 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.

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WEDNESDAY MORNING PLENARY LECTURE

45.0

PHENOTYPIC AND EVOLUTIONARY ADAPTATION. A.F. Bennett. Dept Ecol & Evolut Biol, Sch Biol Sci, Univ California, Irvine, 92717-0001

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 organisms following 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 respective environments. In some instances, features may arise during evolution by such factors as pleiotropy or drift and have no associated benefit. Little comparable attention or discussion, however, has been directed to considering whether phenotypic responses to environmental change may likewise necessarily be assumed to be beneficial and thus true "phenotypic adaptations". 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.

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46.1

PRINCIPLES OF COLD HARDINESS IN ECTOTHERMS. Richard E. Lee, Jr.* Department of Zoology, Miami University, Oxford, Ohio 45056.

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 insuring 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 coelom 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.

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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 (SCPs). 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 intra-intestinal or intra-cellular INAs by securing 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.

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46.3

Fish Antifreeze Proteins

Arthur DeVries Department of Physiology, University of Illinois,Urbana, Il. 61801

The survival of marine fishes in freezing seawater (-1.9°C) is linked to the presence of high levels (25mg/ml) of blood born antifreeze (AF) proteins. The AFs are either glycopeptides (AFGPs) or peptides (AFPs). 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 AFs constitutively throughout the year while many northern fishes regulate levels in response to seasonal temperature changes. Althoughl 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 AFGPs composed of the basic repeating glycotripeptide unit $(Ala/Pro\text{-}Ala\text{-}Thr)_n$ with the disaccharide, galactosyl-N-acetylgalactosamine attached to the Thr's and are present in at lease 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 pouts which are largely random in amino acid composition and in contrast to all others have a compact structure with a molecular weight of 7Kd.. The AFs adsorb to specific ice crystal planes which vary with AF type. Apparent lattice matches have been identified for both the AFGPs and the helical peptides. The mechanism of ice growth inhibition results from increases in local surface curvature 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, ocular and endolymph) lack AFs and remain supercooled because the tight capillaries are barriers to the passage of blood born "growth inhibited crystals".

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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, centipedes). In most of these freeze avoiding species THPs function as antifreezes by inhibiting inoculative 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 that from the beetle Dendroides canadensis. An interesting feature of the sequence of this ~8kDa 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 phytogenetic 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 ~24 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.

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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 directional solidification and cryomicroscopy, organ-specific features of freezing have been identified in liver, heart, and skeletal muscle of frogs and turtles including the importance in 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 desiccation resistance in amphibians and that some of the metabolic events of freeze tolerance, such as cryoprotectant synthesis, are triggered as responses to cellular dehydration. Recent studies of the regulation of cryoprotectant 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 glycemic 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. Of the lesions identified to date (expansion-induced lysis, lamellar-to-H_{II} phase transitions, and the fracture-jump lesion), all are a consequence of freeze-induced dehydration (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-H_{II} 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)

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47.1

NEUROPEPTIDES AND BEHAVIORAL COORDINATION IN HYDROSTATIC ORGANISMS. Ian D. McFarlane*, Diane Hudman*, and Kwangwook Cho*
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 this bag can adopt such an amazing variety of shapes and indulge in a wealth of complex behavioural responses. 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: 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 nerve net portrayed in standard texts. Earlier work showed that the nervous system is made up of at least three separate, but interacting, conducting systems and the work of Grimmelikhuijzen and colleagues has shown that individual neurons in the nerve net can express any one of a dozen or more neuropeptides: Antho-RFamide, Antho-RWamide I and II, Antho-RIamide, Antho-KAamide, Antho-RNamide, Antho-RPamides I to V, and Antho-KPPamides I to III. Almost every neuropeptide identified in sea anemones has a physiological action on one or more muscle groups. What lessons can be learnt about the organisation of neuromuscular systems in a hydrostatic organism? First, multiple neuropeptides are involved even in these simple animals. Some may act directly on muscles, others may act on neuronal pacemakers. Secondly, antagonistic muscles may show opposite responses to a given neuropeptide, one group being inhibited and the other stimulated. This may be a basic rule in situations where antagonistic muscles are in close proximity and diffusion of transmitter is possible. Thirdly, inhibition of spontaneous contractions is an important way in which shape changes can be produced. A new technique allows us to study the action of these neuropeptides on single myoepithelial cells from the body wall. Results confirm that some neuropeptides have opposite actions on antagonistic muscle groups.

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47.2

MULTIPLE NEUROPEPTIDES, REGULATED BY DIFFERENTIAL RNA PROCESSING, MODULATE CARDIORESPIRATION IN *LYMNAEA*.
Niovi Santama, Kingsley J. A. Cox, Paul R. Benjamin and Julian. F. Burke.
Sussex Centre for Neuroscience, The University of Sussex, Brighton BN1 9QG, U.K.

Some anatomically and physiologically well characterised neuronal networks of molluscs provide useful model systems for analysing the role of neuropeptides in neuronal signalling. In the pulmonate snail *Lymnaea stagnalis*, the integrated networks controlling respiration and heartbeat utilise neuropeptides encoded by the large multi-exon FMRFamide locus. The tetrapeptide FMRFamide and structurally related peptides are ubiquitous in *Mollusca* and are widely distributed in the major invertebrate phyla. In *Lymnaea*, the FMRFamide gene encodes for 13 putative neuropeptides, most of which were previously unknown and have now been confirmed by biochemical or biophysical methods[1]. Alternative splicing of the primary FMRFamide RNA transcript in *Lymnaea* generates 2 distinct mRNAs that are differentially expressed in the CNS in a mutually exclusive manner. Post-translational processing of each mRNA liberates two non-overlapping sets of peptides that are differentially distributed in the CNS. Such post-transcriptional/translational mechanisms determine neuropeptide identity and distinct neuropeptide distribution in the CNS and in particular in identified neurons of the cardiorespiratory network, such as the E_{he} cardioexcitatory motoneurons and the visceral white interneuron, VWI. We are beginning to understand the transmitter-like properties of some of the peptides (e.g. FMRFamide, EFLRIamide, GDPFLRFamide) or their combined modulatory functions (e.g. FMRFamide and "SEEPLY") in the central and peripheral targets of the cardiorespiratory network[2]. Recent cloning and functional characterisation of G protein-coupled receptor cDNAs from the CNS of *Lymnaea*[3] and identification of their ligands will enable us to dissect in more detail the signalling mechanisms mediated by neuropeptide transmitters.

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47.3

LOCUSTS AS MODELS FOR THE STUDY OF NEUROPEPTIDES

Graham Goldsworthy, Birkbeck College, University of London, London WC1E 7HX, UK.

Locusts have proved to be extremely good systems in which to explore most aspects of neuropeptides research. Studies involved in the identification, characterization, synthesis, secondary structure, and modes of action of neuropeptides such as the adipokinetic and diuretic peptides[1,2], have been strong themes in locust research. The African migratory locust, *Locust migratoria*, synthesizes three adipokinetic hormones: a decapeptide and two octapeptides. The mechanism of adipokinetic hormone synthesis has been elucidated in locusts of another genus (*Schistocerca*) which produces only the decapeptide and one (different) octapeptide. All three of the *Locusta* adipokinetic hormones have similar overlapping activities. The detailed dose-response and structure-activity relationships of these adipokinetic hormones have been elucidated for three of their actions: lipid mobilization *in vivo*, inhibition of acetate uptake into fat body *in vitro*, and inhibition of RNA synthesis in fat body. These studies suggest most strongly that there are different and changing populations of receptors for these peptides during adult development[2]. Unfortunately, at present receptors for the adipokinetic hormones have not been characterized because of the difficulty of obtaining a biologically active probe of sufficiently high specific radioactivity. The major diuretic peptide in the locust is a 46 amino acid peptide. Structure-activity studies on such a large peptide are more difficult than with the adipokinetic hormones, but receptor studies for this peptide are underway. More rapid progress has been made in studies of receptors for other diuretic peptides, the achetakinins[1] of the cricket *Acheta domesticus*, a near a relation of the locust. The much smaller achetakinins have proved more readily amenable to structure-activity and receptor studies. A high specific activity biologically active probe for these peptides has been developed, and preliminary characterization of achetakinin-binding sites on plasma

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47.4

CRAB NEUROPEPTIDES: MULTIFUNCTIONAL AND MULTIHORMONAL ROLES IN PHYSIOLOGICAL INTEGRATION. Simon Webster. Sch Biol Sci, Univ of Wales, Bangor, Gwynedd LL57 2UW, 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 (VIH) 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 a s moulting and reproduction (Webster, 1991, 1993). Our recent discovery (Wainwright, 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 ecdysteroid production and vitellogenesis, it seems reasonable to speculate that crustacean moulting and vitellogenesis are ultimately **negatively** regulated a complex interaction of several neuropeptides.

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47.5

MODULATORY ACTIONS OF PEPTIDES IN THE FEEDING BEHAVIOR OF *APLYSIA*: CELLULAR MECHANISMS AND FUNCTIONAL IMPLICATIONS K.R. Weiss, V. Brezina, E. Cropper, J. Heierhorst, W. Probst, F. Vilim & I. Kupfermann. Dept. Physiol., Mt. Sinai Schl. Med., NY

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 modulation of the relationship between contraction amplitude and relaxation rate, the two parameters that determine the duration of contractions. Since the cholinergic motorneurons of the feeding musculature 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 cAMP independent mechanisms. 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 relaxation rate is mediated via cAMP, and appears to involve the phosphorylation of the myosin associated protein, twitchin, which through its own kinase domain may modulate myofilamentous proteins.

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47.6

REGULATION OF THE *flp-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(Phe-Met-Arg-Phe-NH_2)-like neuropeptides in the nematode *Caenorhabditis elegans*. About 30 neurons, or roughly 10% of the nervous system in *C. elegans*, stain with an anti-FMRFamide antiserum. Seven putative FMRFamide-related peptides, all containing an N-terminal FLRFamide, are encoded by two transcripts of the *flp-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 serotonin in an egg-laying assay.

To examine the transcriptional regulation of the *flp-1* gene, we have used *lacZ* as a reporter gene under the transcriptional control of varying fragments of the *flp-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 332 bp of the start site of transcription. Deletion analysis on this region is being performed to map this element more precisely.

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

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48.1

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 *Metapeneus 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.

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48.2

CARDIOVASCULAR DEVELOPMENT IN FISHES. Peter J. Rombough. Fac of Sci, Dept Zool, Brandon Univ, 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 about 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 blood pressure, blood pO_2, 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 embryos 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. O_2 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 be reflected in the embryos. However, these manifold heart morphologies all arise from the fusing of simple cardiac tubes. At what point do the developmental trajectories of these different morphs 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 to hypoxia. In contrast, in kingsnake 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 4 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.

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48.5

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 chorioallantoic membrane expands to encompass the embryo and contents of the egg with development. The outer surface of the chorioallantoic membrane is well vascularized for gas exchange, and the mixed oxygenated and deoxygenated bloods empty into it through the intra- and extra-cardiac shunts. We review blood circulation in late chicken embryos with regard to the cardiovascular system and shunt (1). As embryos grow, total blood volume, stroke volume, cardiac output, blood flow through the chorioallantoic gas exchanger and arterial blood pressure increase with embryonic development. However, developmental changes in heart rate are not correlated with increases 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.

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48.6

FUNCTIONAL DEVELOPMENT OF THE CARDIOVASCULAR SYSTEM IN MAMMALS.
Kent Thornburg, Mark Reller, George Giraud and Mark Morton
Oregon Hlth Sci Univ., Depts 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 myofibrills, mitochondria and less sarcoplasmic reticulum than adult myocytes. Contraction is highly dependent on trans-sarcolemmal Ca^{2+} fluxes. A larger fetal than adult myocyte surface area/volume ratio allows normal contraction in spite of the transmembrane Ca^{2+}requirement. Coronary flow at maximal conductance is greater in fetal than adult hearts allowing comparable O_2 deliveries in a hypoxemic mileau. Myocytes normally grow by cell division in early embryo life. In rats, myocytes terminally differentiate after birth and grow only by hypertrophy. In sheep, this occurs mid-gestation. Pressure loading alters hemodynamic function, alters myocyte differentiation and accounts for mal-adaptive growth with outflow tract obstruction. The fetal heart works within the framework of the passive pressure-volume relation and end systolic-dimension relationship but high rates impair filling. In sheep, right side stroke volume increases at birth by 50% and left side by 100% as predicted by mechanical interaction.

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

49.2

Renal Sodium Cotransport Systems: Diversity and Evolution
R.K.H. Kinne, A.I. Morrison-Shetlar*, H. Kipp, Ch. Bevan+ and E. Kinne-Saffran
Max-Planck-Institut für molekulare Physiologie, Dortmund, FRG and Mount Desert Island Biological Laboratory, Salsbury Cove, ME, USA; *Wesleyan University, Middletown, CT, USA; +EXXON Biomedical Sciences, Inc., East Millstone, NJ, USA
Supported by NSF EPSCoR Grant # EHR-9108766

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 membrane vesicles, immunological techniques and cloning strategies the sodium-D-glucose cotransport systems in hagfish (*Myxine glutinosa*), shark (*Squalus acanthias*), skate (*Raja erinacea*), toadfish (*Opsanus tau*) and flounder (*Pseudopleuronectes americanus*) kidneys were investigated. They differ in their phenotype with regard to substrate specificity, affinity to inhibitors and the number of sodium ions translocated. Immunotyping 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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49.3

Chloride Secretion by the Rectal Gland: Lessons from the Shark

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

The salt-secreting 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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49.4

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 rat atrial natriuretic peptide stimulated 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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49.5

RENAL SECRETION IN GLOMERULAR AND AGLOMERULAR FISH.

Klaus W. Beyenbach. Section Physiology, Cornell Univ., Ithaca, N.Y. 14853.

There are some 30 species of aglomerular fish which do not use glomerular filtration and tubular reabsorption as the dominant renal two 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 urines of glomerular and aglomerular 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 aglomerular 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 aglomerular fish also secrete fluid in vitro, as expected. Rates of fluid secretion and the composition of fluid secreted by aglomerular proximal tubules are strikingly similar to those measured in glomerular proximal tubules, suggesting similar mechanisms of tubular secretion in aglomerular and glomerular proximal tubules. Central to the epithelial secretion of salt and water in aglomerular 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 hyperosmolarity, and for the downstream flow of tubular fluid.

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49.6

PISCINE PASSING OF PROTONS: ACID-BASE AND ION REGULATION BY FISH KIDNEY AND BLADDER. Chris M. Wood, Biology, McMaster U., Hamilton, Canada L8S 4K1.

The importance of the renal system in freshwater fish is often discounted relative to the gills because of the low concentrations of NaCl 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 3-4x their unidirectional 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 NH_4^+ output increases greatly and titratable acidity ("TA"; mainly phosphate) only moderately. During respiratory acidosis induced by environmental hyperoxia, renal NH_4^+ output increases to a lesser extent and TA output to a greater extent, and the rate of H^+ secretion (= HCO_3^- reabsorption) in the renal tubules is comparable to that at the gills (1). The enzymatic basis of the differences in renal ammoniagenesis between the two types of acidosis is under investigation. Embryologically, the urinary bladder is part of the renal system; *in vitro* studies suggest it has a capacity for urine modification, but traditional urine collection techniques bypass the bladder. The recent development of an external catheterization technique which allows the bladder and its sphincters to function normally has revealed that urination is periodic in trout (2). During the 25 min storage period in the bladder, electrolytes are scavenged from the urine, thereby increasing renal effectiveness. Urinary NaCl content is reduced by about 50%, K^+ by about 40%, and volume by about 25%; acid-base modification appears minimal (3). (Supported by NSERC).

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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 mounted in Ussing chambers have provided a means to characterize the mechanisms and regulation of several transepithelial transport processes. Electrophysiological measurements show that the cultures maintain proximal tubule-like properties, i.e., low transepithelial potential difference (-0.6 ± 0.10 mV), resistance (23 ± 2.3 ohms x cm^2) and short-circuit current (24 ± 2.7 $\mu A/cm^2$). Transepithelial polarity is dependent on the direction of the NaCl concentration gradient and indicates that sodium is about four times more permeant than chloride (1). Apical plasma membrane potential difference is about -60 mV and due largely to the K^+ diffusion potential (2). Confluent monolayers perform net active transepithelial reabsorptiion (lumen to peritubular side) of glucose (including the analog, α-methyl glucoside) and net active secretion of *p*-aminohippuric acid, taurine, and sulfate. Inorganic phosphate may undergo either net reabsorption or net secretion. Simulation of metabolic acidosis stimulates P_i secretion through a protein kinase c dependent process whereas treatment with stanniocalcin or somatolactin stimulates reabsorption, and both hormones are protein kinase A dependent. The hydrophobic P-glycoprotein substrate, daunomycin, is actively secreted. This process is enhanced approximately two-fold by sublethal heat shock and is inhibited by vinblastine and cyclosporine A (3). Tissue-level function in culture has thus provided the bases for certain predictions concerning function of the intact kidneys. Supported by NSF.

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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 Res. Inst., Univ. Tokyo, Tokyo 164 and Dep. Biol. Sci., Tokyo Inst. Technol., Kanagawa 227, Japan

Natriuretic peptide was first identified in mammalian atria and is now known to form a peptide family consisting of at least A-type (ANP), B-type and C-type (CNP) natriuretic peptides. In bony fish, ANP was isolated from eel atria and CNP from killifish and eel brains. In addition, a new type of peptide named ventricular natriuretic peptide (VNP) has been isolated from eel and trout ventricles. cDNAs of eel ANP and VNP have been cloned. Northern analysis revealed that ANP is expressed in atria and VNP most abundantly in ventricles.

ANP secretion transiently increases after transfer of eels to seawater, but plasma levels of seawater-adapted eels are not altered because of increased clearance rate. Principal stimulus for ANP secretion in the eel is increased plasma osmolality rather than increased blood volume. The latter is the major stimulus for ANP secretion in mammals. ANP inhibits drinking and intestinal sodium and water absorption in the eel as in mammals, but it unexpectedly increases cortisol secretion and inhibits urine flow.

Three types of natriuretic peptide receptors (NPR) have been identified in mammals; NPR-A and NPR-B which have guanylyl cyclase domain intracellularly, and NPR-C which lacks the intracellular domain. ANP receptors have been localized in the eel gill, kidney and other tissues by autoradiography. cDNAs for eel NPR-B and NPR-C have been cloned. RNase protection analysis and ligand binding analysis revealed that expression, capacity and affinity of these receptors are altered after transfer of eels to seawater.

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50.1

STRATEGIES OF ANTIOXIDANT DEFENSE
Helmut Sies, Institut für Physiologische Chemie I
Heinrich-Heine-Universitat Dusseldorf,
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 antioxidants mimic biological strategies, e.g. the selenoorganic compound ebselen as a GSH peroxidase mimic (3).

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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 & Spicer, 1988). During moderate hypoxia, oxygen consumption (Mo_2) is maintained over a range of Po_2 until a critical Po_2 (Pc) is reached below which Mo_2 declines sharply and anaerobic metabolism becomes increasingly important. Values for the Pc appear to be correlated with the degree of hypoxia normally experienced; the lowest Pc values are shown by species experiencing more extreme conditions. The respiratory mechanisms by which Mo_2 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 haemolymph 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 affecting oxygen affinity have made a significant contribution to our understanding of the physiological mechanisms operating during exposure to hypoxia (Morris, 1990). Long-term exposure to moderate hypoxia may result in the modification of some of these respiratory responses and allows the possibility of invoking additional responses such as an increase in HCY concentration and alterations of the subunit composition of the HCY which may increase oxygen affinity.

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50.3

MULTIPLE FORMS OF ANAEROBIOSIS: ENVIRONMENTAL AND SULPHIDE DEPENDENT ANAEROBIOSIS.
Manfred K. Grieshaber, Institut für Zoologie, Lehrstuhl für Tierphysiologie, Heinrich-Heine-Universitat, 40225 Dusseldorf, 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 ambient Po_2 may be kept constant in a wide range of Po_2 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 Po_2 (P_c), physiological mechanisms are insufficient to augment an aerobic energy metabolism. Below this P_c anaerobiosis commences. The P_c 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 abiogenic ecological factors such as temperatur, salinity changes or sulfide. The exposure to sulphide shifts the P_c to higher Po_2 values and energy provision is reduced due to an sulphide induced anaerobiosis. Energy is mainly provided via the same pathways as during environmental anaerobiosis.

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50.4

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

Erich Gnaiger[a], Gabriela Méndez[a], Steven C. Hand[b]
[a]Department of Transplant Surgery, Clin. Interdisc. Bioenergetics, University Hospital of Innsbruck, A-6020 Innsbruck, Austria; [b]Department of EPO Biology, University of Colorado, Boulder 80309-0334 USA

Within tissues, mitochondria are protected from high atmospheric O_2 levels and high O_2 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 p_{50} 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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SCHOLANDER AWARD BANQUET LECTURE

51.0

THE SCHOLANDER LEGACY: FROM SIMULATED DIVING TO MICROCOMPUTERS ON MESOPOLAGIC SEALS. P.W.Hochachka, Dept. of Zoology, University of B.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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9.1

THE EVOLUTION OF METABOLIC RATE IN LARVAL DROSOPHILA. David Berrigan, J. Mcabe*, and L. Partridge*. Department of Zoology NJ-15, Univ. of Washington, Seattle WA, 98195, USA, and Department of Genetics and Biometry, University College London, 4 Stephenson Way, London NW1 2HE, 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 divergence. The 16.5 °C (and the higher latitude) flies grow faster and have shorter larval development times than the 25 °C (and the more equatorial) flies, regardless of developmental temperature. Increased growth rates could be a result of increased growth efficiency due to decreased routine 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 18 or 25 °C for two generations 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 lines than in the 25 °C lines 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 allometry 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*) Jill B.K. Leonard and Stephen D. McCormick. Conte Anadromous Fish Research Center, National Biological Survey, Turners Falls, MA and Department of Biology, University of Massachusetts, Amherst, MA, USA.

American shad is the most abundant anadromous fish on the eastern coast of the U.S. Due to the increasing use of fish ladders to mitigate manmade barriers to migration, it is of interest to understand the pattern of energy utilization as fish move upstream. Twenty adult shad (10 male; 10 female) 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 hematocrit did not, indicating an enrichment of the hemoglobin within red blood cells rather than a proliferation of cells. Cardiosomatic index increased significantly between 1 and 228 km in females, but not males. Citrate synthase activity decreased in liver and red muscle (but not in white muscle) between 1 and 228 km. Alanine aminotransferase (GPT) activity increased markedly in liver in both sexes and in female white muscle. In the red muscle, GPT was highest in both sexes at the 139 and 198 km sites. β-Hydroxyacetyl coenzyme A dehydrogenase (HOAD) also showed an activity peak at the intermediate sampling points in both sexes in the liver and white muscle. There was no change in red muscle HOAD activity. Stored tissue glycogen is quickly metabolized in the lowest reach of the river. There was no change in total lipid content in the liver of females while in males there were significant, though variable, differences with fish just entering the river having the highest levels. Muscle lipid levels were similar in both sexes and dropped as they ascended the river. In both sexes, the largest usage of red muscle lipid on a per kilometer basis was between 139 and 198 km while use of white muscle lipid was greatest between 198 and 228 km. Both these river reaches contain upstream fish passage structures, but other factors may be involved. The cost of migration for these migratory fish is not a evenly distributed over the migratory route nor are males and females using energy reserves in the same pattern.

9.3

THE TEMPERATURE DEPENDENCE OF INTRACELLULAR pH IN FROG SKELETAL MUSCLE. M. Marjanovic, A.C. Elliott, B.B. Roman, and M.J. Dawson. University of Illinois, Urbana-Champaign, IL 61801.

The temperature dependence of intracellular pH (pH_i) was studied using ^{31}P-NMR in isolated gastrocnemius muscle. Between 4° and 37°C, pH_i decreased linearly as the temperature rose, tracking the pH of neutrality (i.e. $H^+=OH^-$) as previously described by Malan, Wilson and Reeves (*Respiratory Physiology* 28:29-47, 1976) and by Elliott and Dawson (*Journal of Physiology* 360:59P, 1985). A model solution containing the buffers present in the cytosol showed the same temperature dependence as the muscle. Inhibition of anaerobic metabolism had no effect, demonstrating that the temperature dependence of lactic acid production does not account for the temperature dependence of pH_i. The temperature dependence of pH_i was the same whether the extracellular pH or H^+/OH^- was maintained constant. Stimulation of the Na^+-H^+ exchanger using insulin resulted in intracellular alkalization which was temperature-independent. These studies confirm that the temperature dependence of pH_i is property of the muscle itself and is not a consequence of blood acid-base regulation. We suggest that when the temperature is changed, a new pH_i is immediately achieved due to the temperature-dependence of the pKs of the intracellular buffers. Long-term maintenance of pH_i near neutrality suggests that the set-point of the Na^+-H^+ exchanger must also vary with temperature.

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9.4

DIET, HIBERNATION, AND THE ANTIOXIDANT DEFENSES OF GROUND SQUIRRELS. Craig L. Frank. Fordham University, Armonk, N.Y. 10504

Ground squirrels (Spermophilus lateralis) 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 diets is the increased production of toxic lipid peroxides. Linoleic acid is 12 times more likely to produce lipid peroxides than other fatty acids. Mammals have several antioxidant enzymes that serve 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 S. lateralis. The results of these experiments reveal that in brown adipose tissues, 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. Blair O. Wolf and Glenn E. Walsberg. Department of Zoology, Arizona State University, Tempe, AZ 85287-1501.

Small birds, because of their small body mass and high surface area to volume ratios are tightly coupled to the physical environment. We examined effects of solar radiation, and wind speed (0.4 - 3.0 m s^{-1}), and their interaction, on metabolic rates in the Verdin, *Auriparus flaviceps*, a very small (7.1 gm), desert-dwelling bird. Exposure to solar radiation significantly reduces metabolic power consumption at all wind speeds measured except 3.0 m s^{-1}. At an irradiance of 1000 W m^{-2} and a wind speed of 0.4 m s^{-1} metabolic rate may be reduced by 58%, equivalent to 2.3 times basal metabolic rate. Solar heat gain at 3.0 m s^{-1} reduces metabolic rate by only 4%. Thus, changing wind speed from 0.4 to 3.0 m s^{-1} reduces solar heat gain by 90%! These changes can be equated to changes in air temperature by calculating standard operative temperatures. Exposure to solar radiation at a wind speed of 0.4 m s^{-1} results in an elevation of T_{es} of 18 °C. As a result, a Verdin that spends 90% of its day foraging during the winter may shift from a cold microclimate (15 °C) to a thermoneutral microclimate by simply moving out of the shade and into the sun. In contrast, during the summer when T_{es} may reach 60 °C, midday activity is severely suppressed and foraging occupies less than 25% of each hour. At that time, water rather than energy may constrain behavior. Under these conditions, Verdins can reduce rates of evaporative water loss by 5 to 10-fold by occupying shaded microclimates. These results highlight the importance that subtle changes in microclimate can have on the water and energy balance in a small bird.

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.

Heat 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 HL rhythm relationships than in LD. Whole body HP, HL, activity and BT were measured for 7 days in 5 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 for a given animal, indicating close coupling among rhythms. Individuals showed 24.7 to 25.7 hour periodicity. Like entrained animals, elevated BT during the active period (α) was accompanied by elevations in both HP and HL. Changes in HL lagged behind those of HP at the start of α and rest (ρ) periods, when BT changes were greatest. Average HP and HL minima were ca. 3 W/kg and maxima between 5.5 and 6 W/kg. Changes in BT, HP, and HL between α and ρ were less abrupt in LL than in LD. Activity bouts during ρ, with increased HP and HL, were more common in LL than in LD, but HL increases maintained low BT despite additional activity. Coordinated HP and HL rhythms produced the observed BT rhythm in LL as in LD, although wave forms differed.

9.7

METABOLIC ADAPTIVE STRATEGIES OF DRY AND WET ADAPTED ZEBRAFINCHES *(TAENIOPYGIA GUTTATA)* ON LOW AND HIGH FAT DIETS. Margot Mager and Günther Warncke. Institute of Neurophysiology of the University of Cologne, Robert-Koch-Str.39, 50931 Cologne, Germany.

Zebrafinches are living in arid zones. It is uncertain, how and to what extent water budget and nutrition, above all the fat content of the food, influence the adaptation to extreme environment. It is also not clear, whether this has an effect on thermoregulation, muscle activity and energy balance. As zebrafinches are well adjusted to their xerotic environment, one can presume that they are able to produce strongly concentrated urine and minimize their water loss by reduced evaporation.

We investigated four groups (n=15) of zebrafinches under laboratory conditions (LD 12:12, ambient temperature: daytime, 25°C; nighttime, 18°C): 1: low fat dry (l-d), 2: low fat wet (l-w), 3: high fat dry (h-d), 4: high fat wet (h-w). For six month the birds had been adapted to wet (water ad lib.) and dry conditions (15 min water ad lib. every 10 days) on low and high fat diets. Thereafter the total water intake (preformed and oxidative water, water ad lib.) and the total water loss (evaporation, faeces, urine) was evaluated in order to calculate the daily water balance (see table).

diet	l-d	l-w	h-d	h-w
total water intake	**1,47 g**	**4,14 g**	**1,58 g**	**3,47 g**
water ad lib.	0 g	2,67 g	0 g	2,04 g
oxidative water	1,19 g	1,00 g	1,34 g	1,11 g
preformed water	0,29 g	0,25 g	0,25 g	0,21 g
total water loss	**1,96 g**	**4,13 g**	**1,19 g**	**3,07 g**
cloacal water loss	0 g	0,57 g	0 g	0,31 g
evaporation	1,77 g	3,25 g	1,02 g	2,42 g
water loss by faeces	0,19 g	0,35 g	0,18 g	0,37 g
water balance	**– 0,49 g**	**0,01 g**	**0,39 g**	**0,40 g**

Investigations of blood parameters of all four groups differed significantly in red blood count, haematocrit, haemoglobin, blood urea nitrogen, uric acid, triglycerides and cholesterol. The content of fatty acids, over alll the amount of free fatty acids, is still being evaluated as well as the electromyograms, calorimetry of faeces and kidney-histology.

The results so far show that dry adapted zebrafinches can reach homeostasis in their water balance by increasing food intake, reinforced intestinal reabsorbence of water, decreasing evaporation, reducing the part of bloodplasma in full blood and by concentrating the urine and blood.

A high fat content seemed to be advantageous.

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9.8

EFFECTS OF CO_2 AND ATP ON O_2 BINDING BY TURTLE ISOHEMOGLOBINS AT 30°C. John G. Sikora* and Leigh A. Maginniss. Dept. of Biological Sciences, DePaul University, Chicago, IL 60614.

IsoHbs 1 and 2 from the western painted turtle (Chrysemys picta) were separated, purified and prepared in physiological medium (Hb_4 = 1 mM). Isocapnic oxygen equilibrium curves (O_2EC) were generated for isoHb solutions at 1, 2 and 3% CO_2 and 30°C using thin film techniques. Half-saturation PO_2 (P_{50} at pH 7.6), CO_2 Bohr effect ($\Delta\log P_{50}/\Delta pH$) and Hill slopes (n) for two saturation ranges are presented (mean ± SEM).

Treatment	P_{50} (Torr)	CO_2 Bohr Effect	Hill's n 0.2–0.4 S	Hill's n 0.6–0.9 S
Hb 1	5.3 ± 0.4	–.20 ± .14	3.0 ± 0.2	7.3 ± 0.4
Hb 2	9.5 ± 0.5	–.53 ± .07	2.2 ± 0.1	4.9 ± 1.1
Hb 1 + ATP	5.9 ± 0.4	+.10 ± .05	2.4 ± 0.2	5.6 ± 0.5
Hb 2 + ATP	12.5 ± 1.0	–.63 ± .10	2.1 ± 0.1	3.9 ± 0.2

Curvilinear Hill plots with n values exceeding 4 may reflect isoHb aggregation. ATP (2 mM) reduced cooperativity for both isoHbs. O_2 affinity for isoHb 1 was temperature-insensitive; $\Delta\log P_{50}/\Delta$°C was 0.002 in the absence of ATP. Corresponding $\Delta\log P_{50}/\Delta$°C for isoHb 2 was 0.012. O_2EC shapes for isoHb mixtures (75% Hb 1 + 25% Hb 2) were different from those predicted from the O_2 binding results of the two individual isoHbs. These findings may represent functional interaction between Hb 1 and Hb 2. (Supported by DePaul University LA&S and URC grants and Grant-in-Aid of Research from Sigma Xi.)

9.9

A MODEST FRACTION OF TIDAL VOLUME IS STEP-RELATED IN TROTTING DOGS. U. Silke Birlenbach, Robert B. Banzett*, Stephen H. Loring*, and David R. Carrier*. 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" and ribcage, ribcage-loading by the forelimbs, and spinal flexion. To estimate the extent of the mechanical linkage, we determined the step-related volume changes (V_S) in relation to tidal volume (V_T). In 2 dogs (~20 kg) trotting at at 2-2.8 m/s, respiratory airflow ($\dot{V}$) 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, 1:2 to 1:9 (br:step). The phase relationship of locomotion and respiration drifted sometimes. When steps occured randomly throughout respiratory cycles, we averaged ventilatory volumes (containing V_T and V_S) over many step periods: volume changes related to breathing (V_T) averaged to 0, and only V_S remained. V_S ranged between 3 and 16% of V_T. Some of this could be artifact (mask movement), judged from apparent $\dot{V}$ in phase with locomotion during swallowing. We conclude that the mechanical effect of locomotor events on V_T is modest. Other step-related effects not detectable by this approach, eg. intrapulmonary gas mixing, are possible. Support: HL 35420, NSF IBN9306466, Beth Israel Anaesthesia Found.

9.10

OPEN-FLOW PLETHYSMOGRAPHY: A NEW APPROACH TO MEASURE Vt. J.G. Chaui-Berlinck* and J.E.P.W. Bicudo, Department of Physiology, University of São Paulo, Brazil.

In order to calculate tidal volumes (Vt) in an open-flow plethysmography system the usual procedure is to inject a known volume of air inside the animal chamber and correlate the deflection obtained with deflections caused by the animal (accounting for temperature and humidity effects) (Malan, A., Respir. Physiol., 17:32-44, 1973), or integrate the signals from the animal with respect to time. However, both procedures are misleading, basically because (1) the deflection is not caused by the injected volume (or Vt), but by the injection flow (or ∂ Vt), and (2) integrating such signal would introduce errors because the pressure inside the chamber usually does not reach the baseline before the animal starts to expire. To solve this problem an exponential equation correlating the outlet ($\dot{Q}o$; dependent variable) to the inlet flow ($\dot{Q}i$; independent variable) as a function of time, taking into account the system escape constant J, was empirically introduced. $\dot{Q}o$ and $\dot{Q}i$ are integrated with respect to time, and the difference between the two values gives the pressure signal at time t. The pressure signal is, therefore, a non-linear function of time and is determined by $\dot{Q}i$ and J. Because the signal produced by the animal inspiration is short, not allowing pressure stabilization, one must calculate this pressure, which is attained when t tends to infinity. Using this approach one is allowed to calculate $\dot{Q}i$ (or ∂ Vt) with great accuracy that otherwise would not be possible, unless Vt is known beforehand. Supported by FAPESP, CNPq and CAPES, Brazil.

9.11

GAS EXCHANGE AND WATER BALANCE IN TENEBRIONID BEETLES: ROLE OF THE SUB-ELYTRAL CAVITY. Michael C. Quinlan* and John R. B. Lighton Department of Biology, University of Utah, Salt Lake City, Utah 84112.

In many tenebrionid beetles, the wing covers (elytra) have become fused to form a chamber covering the dorsal surface of the abdomen. This chamber, the sub-elytral cavity (SEC), also covers the abdominal and metathoracic spiracles and is thought to reduce water loss associated with gas exchange. By treating the SEC as a "natural" respirometry chamber and using flow-through gas analysis, we have examined the respiratory physiology and water relations of the xeric tenebrionid *Eleodes armata*. Two respirometry systems were used in parallel so that CO_2 emission (V_{CO2}) and water loss (WL) from the intubated SEC could be separated from that of the general body surface. Unlike many arthropods that release CO_2 in bursts, *E. armata* excretes CO_2 continuously from the SEC. Intermittent pressure fluctuations were measured in the SEC and may represent ventilation movements. Approximately 85 % of the total V_{CO2} occurred from the spiracles opening into the SEC, and water loss from the SEC was tightly correlated with CO_2 loss. In contrast, external WL and V_{CO2} were not correlated due to the large cuticular component of water loss. Surprisingly, external V_{CO2} from the mesothoracic spiracles and CO_2 loss from the SEC were not closely coupled.

9.12

VAGAL FEEDBACK AND VENTILATION DURING URETHANE "SLEEP" AND HIBERNATION. M.B. Harris* and W.K. Milsom. University of British Columbia, Vancouver, BC. V6T 1E3.

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 voltage, high frequency, state I) and slow-wave-sleep-like (high voltage, low frequency, state III) EEG states of urethane anesthesia as well as during hibernation in unanesthetized animals before and after vagal blockade. Tidal volume (VT) increased while respiratory frequency (fR) and minute ventilation ($\dot{V}$E) decreased from state I to state III and from euthermia to hibernation. During hibernation breathing became episodic. On exposure to 4% CO_2, $\dot{V}$E increased in all cases. In hibernation this was due to an increase in fR while, in all other states it was due primarily to an increase in VT. Vagal blockade reduced fR and increased VT in all cases, yet had a negligible effect on VE. Breathing no longer changed between state I and state III suggesting that the change in breathing seen in intact animals may have been due to a reduction in vagal tone. In hibernation, however, VT still increased and breathing still became episodic, although fewer breaths occurred per episode. The responses to CO_2 were unchanged by vagotomy in all cases except hibernation where the response increased. These data indicate that the effects of vagal feedback 1) may be reduced in the slow-wave-sleep-like state (III) of urethane anesthesia but 2) still modulate breathing pattern during situations with reduced respiratory drive.

9.13

VENTILATION - PERFUSION RELATIONSHIPS IN THE SAVANAH MONITOR LIZARD (VARANUS EXANTHEMATICUS). S. R. Hopkins, J.W. Hicks, T. K. Cooper* and F.L. Powell. Department of Medicine, University of California, San Diego, La Jolla CA, 92093-0623 and Department of Ecol. and Evol. Biol., University of California Irvine, Irvine CA, 92717.

The effect of exercise on regional matching of ventilation ($\dot{V}$) and perfusion ($\dot{Q}$) has only been studied in mammals. Lung complexity and aerobic capacity in varanid lizards is high by reptilian standards although less than in mammals. We used the multiple inert gas elimination technique to measure $\dot{V}/\dot{Q}$ heterogeneity in awake Savanah Monitor Lizards (*Varanus exanthematicus*) at rest and during activity. Trace amounts of six inert gases were infused via the external jugular vein. Blood samples were collected from the pulmonary artery and the left atrium and mixed expired gas samples and metabolic data were acquired. Indices of $\dot{V}/\dot{Q}$ heterogeneity were calculated using a 50 compartment model (means ± sd):

$\dot{V}O_2$ (ml/min) STPD	$\dot{V}E$ (ml/min) BTPS	$\dot{Q}$ (ml/min)	SD of $\dot{Q}$ vs log $\dot{V}/\dot{Q}$ distribution	intrapulmonary shunt (% of $\dot{Q}$)	dead space (% of VE)
1.18±0.16	58.2±32.1	89.3±13.1	0.50±0.23	6.90±0.85	40.3±19.0
2.48±1.39	203.2±9.12	145.3±6.1	0.90±0.14	2.35±1.48	14.5±5.1

These data show increasing $\dot{V}/\dot{Q}$ heterogeneity with exercise, similar to that found in mammals.

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9.14

THE SAVANAH MONITOR LIZARD (VARANUS EXANTHEMATICUS) INCREASES VENTILATION DURING EXERCISE. T. K. Cooper*, S. R. Hopkins, F. L. Powell, and J. W. Hicks. Department of Medicine, University of California, San Diego, La Jolla CA, 92093-0623 and Department of Ecol. and Evol. Biol., University of California Irvine, Irvine CA, 92717.

It has been reported that the Savanah Monitor Lizard (*Varanus exanthematicus*) has a severe mechanical limitation during exercise causing a decrease 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 miniature 2-way non-rebreathing valve (Hans Rudolph 2300) 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: (means±sd, n = 4, weight = 1.37±0.25 kg, * $p<0.05$)

Speed (m/s)	$\dot{V}O_2$(ml/min) STPD	$\dot{V}E$ (ml/min) BTPS	idal vol. (ml) BTPS	Frequency (per min.)
0	1.61±0.22	52.0±26.0	14.5±4.4	3.8±2.4
1.65±0.61	7.53±2.61*	502.8±144.0*	7.8±3.6*	74.0±27.0*

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

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9.15

The Effect of Gender and Body Temperature on the Ventilatory Response to Hypoxia in Hooded Rats. Rayna Gonzales and Steve Wood. Cardiopulmonary Physiology Program, The Lovelace Institutes, Albuquerque, NM 87108.

In mammals, acute exposure to hypoxia elicits adaptive responses such as increased ventilation. Hypoxia also induces hypothermia, which may be adaptive when O_2 supply is limited. We examined the potential beneficial effect of hypoxia-induced hypothermia on the ventilatory response of hooded rats to acute hypoxia. We also studied the functional significance with respect to metabolism and blood gas transport. Awake, unrestrained rats were exposed to graded levels of inspired O_2 (1 hr @ 24, 21, 16, 10, and 8%). Ventilation and metabolic rate were measured in: 1) Temperature clamped (TC) rats (T_b kept @ 37° C) and 2) Hypothermic rats (T_b reduced to 33°C). Hypothermic rats had a significantly decreased ventilatory response compared with the TC controls. We found a marked gender difference with higher hypoxic drive in TC females. The ventilatory equivalent (V_E/VO_2) was also increased in the TC rats and more pronounced in females. The mechanism of gender differences was examined with castrated male and peak estrus (minimum progesterone) female rats. Castration increased the hypoxic drive of TC rats and reduced the gender difference. Peak estrus reduced the hypoxic drive of TC females and removed the gender difference. In hypothermic rats there was no significant gender difference, suggesting that hypothermia overrides normal influence of sex hormones on ventilatory response. In conclusion, hypothermia blunts the ventilatory response to hypoxia. However, TC females have an increased hypoxic drive mediated by progesterone. In contrast, TC males have a blunted drive possibly mediated by testosterone. *Research supported by NIH grant HL 40537.*

9.16

MODELLING EFFECTS OF HYDRODYNAMIC CONSTRAINTS ON THE USE OF MORPHOMETRIC CONDITION INDICES IN PINNIPEDS. Brian S. Fadely* and Michael A. Castellini. School of Fisheries and Ocean Sciences, Institute of Marine Science, University of Alaska, Fairbanks, Alaska, 99775.

Morphometric measurements have been used with varying degrees of success as indicators of body condition in pinnipeds. Changes in relationships between mass and a volume index based on length and axillary girth may indicate differences in body condition, but can be affected by changes in body shape. We modelled the effects of hydrodynamic constraints on the limits of potential variability in these condition indices. Data on mass, standard length and axillary girth were collected in the field or gathered from literature for 6 species of phocids and 1 otariid (total n=190). Variability of body girth and ultrasonic blubber depth at 4 locations along the body were measured in 11 adult female Steller sea lions and 17 harbor seals. Results indicated that all animals had length-thickness relationships varying within fineness ratios (length divided by maximum diameter) of 3-5, typical for streamlined animals. That is, relationships between length and maximum diameter, while variable, were within the boundary limits imposed by hydrodynamic consideration. Thus, even though hydrodynamic constraints may impose limits on body shape variation, seasonal or interannual changes in the relationship between mass and volume indices remain inside those limits and can therefore most likely be ascribed to changes in body condition.

9.17

CAPE PANGOLIN: ASSESSING ITS THERMAL ENVIRONMENT IN THE WILD AND ITS IMPACT ON FORAGING ACTIVITY. I. Coulson* and M.E. Heath Sengwa Wildlife Research Institute, Zimbabwe.

The purpose of this study was to define the range of thermal conditions that occur in the Cape pangolin's (*Manis temminckii*) natural habitat, and assess how they influence the above ground foraging activity of this "nocturnal" species that lives in burrows. Six pangolins of 2.8-16.8 kg mass were studied. The full range of ambient temperatures (T_a) available to the pangolin, as well as the T_a immediately next to the pangolin was monitored at 15 min intervals on a 24-hr basis. To this end, a temperature sensitive radio-telemetry transmitter was attached to pangolins externally. T_as were measured in the sun and shade 30 cm above the ground (pangolin height), inside the pangolin's burrow, and at the pangolin's location by the transmitter. During winter and summer, the T_a of the burrow was within the pangolin's thermal neutral zone (TNZ). In winter, the above ground T_as were within the TNZ during the day, but well below the TNZ at night. In summer, the above ground T_as were well above the TNZ during the day, but within the TNZ at night. Therefore, in terms of energy expenditure and thermal stress, the most reasonable time for pangolins to forage would be during the day in winter, and during the night in summer, and remaining in their burrow while inactive. It was found that yearling and subadult pangolins (mass < 8 kg) forage during the day or evening in winter. This allows them to avoid T_as as low as -5°C. In contrast, adult pangolins (mass 8-17 kg) foraged at night throughout the year, but during very cold nights they sometimes refrained from foraging altogether. There was a clear correlation between the above ground T_a during foraging activity and the mass of the individual pangolins.

9.18

PERFORMANCE AND DIGESTIVE FUNCTION OF CEDAR WAXWINGS AND THRUSHES: RESPONSES TO SUGAR CONCENTRATION. Mark C. Witmer. Section of Ecology and Systematics, Cornell Univ., Ithaca, NY 14853

The diet of cedar waxwings is dominated by sugary fruits. Thrushes, like many other avian frugivores, include less fruit in their diets and eat a mix of sugary fruits, fatty fruits, and fatty, proteinaceous arthropods. Sugary fruits contain glucose and fructose and are typically low in protein/amino acids. These fruits vary temporally and interspecifically in water content. I compared digestive function and performance of cedar waxwings and two thrushes (American robin and wood thrush) fed three diets of different glucose (and protein) concentrations, matching the levels of these nutrients in natural fruits. Corroborating results with natural fruits, cedar waxwings showed much higher relative intake rates compared to the thrushes for each diet. All birds showed a compensatory response of intake rate to sugar concentration, resulting in similar dry matter intake rates for the three diets. The response of nitrogen balance to intake suggests that a) cedar waxwings have comparatively low protein needs under these conditions and b) that digestion and/or uptake of protein is less complete for waxwings than for thrushes. Protein deficiency is implicated as a nutritional limitation of sugary fruits for omnivorous frugivores. Cedar waxwings performed well on this high sugar/low protein diet because of high relative intake rates and low protein requirements.

9.19

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 (>6 months), we predicted that their gut would exhibit a much greater response to feeding. We have found that sit-and-wait foraging Burmese pythons (*Python molurus*), 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.

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9.20

PHYSIOLOGICAL EFFECTS OF NEARSHORE NUTRIENT AVAILABILITY ON INTERTIDAL MUSSELS AND THEIR PREDATORS. Elizabeth P. Dahlhoff, 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 rocky-intertidal mussels (*Mytilus californianus* and *M. trossulus*) and their predators (*Pisaster ochraceus* and *Nucella emarginata*) 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 (seastar 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.

9.21

BIOCHEMICAL INDICES OF PHYSIOLOGICAL STATE IN THE MUSSEL *MYTILUS CALIFORNIANUS*. Jonathon H. Stillman and George N. Somero. Dept. of Zoology, Oregon State University, Corvallis, OR 97331-2914.

We have examined the correlations between whole animal oxygen consumption rates ($\dot{V}O_2$), and the activities of malate dehydrogenase (MDH), citrate synthase (CS), 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 $\dot{V}O_2$'s of 0.007 $mgO_2/h \cdot g$, whereas animals measured immediately upon collection had much higher $\dot{V}O_2$'s (0.011). Since freshly collected specimens had very full guts, their high $\dot{V}O_2$'s were likely a result of specific dynamic action. Animals were subsequently held in fed and food-deprived conditions for two months. $\dot{V}O_2$'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 gill, mantle and adductor tissues, CS and MDH activities and RNA:DNA ratios decreased, and PK activity increased in food-deprived mussels. Gill CS, gill RNA:DNA ratios, and adductor RNA:DNA ratios were significantly correlated with $\dot{V}O_2$, but did not explain a large percentage of the variance in $\dot{V}O_2$ ($r^2 \approx 0.20$). Using multiple regression, we created a model for $\dot{V}O_2$ utilizing a combination of enzyme activities and RNA:DNA ratios which explained much more of the variance in $\dot{V}O_2$ ($r^2 \approx 0.70$) 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 maintained elevated metabolic rates for 4 times longer in the winter than during late spring. Increased thermogenic endurance is a common form of seasonal acclimatization among passerine birds, yet its mechanistic basis 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 summit 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 that the physiological mechanism of winter acclimatization in house finches involves increased fat stores, enhanced lipid mobilization, and greater catabolic capacity.

9.23

WEANING MASS DETERMINES HOW STORED FUELS ARE UTILIZED DURING PROLONGED FASTING IN ELEPHANT SEAL PUPS. Lorrie D. Rea* and Michael A. Castellini. Institute of Marine Science, Univ. of Alaska, Fairbanks, AK, 99775.

Plasma concentrations of blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), and β-hydroxybutryrate (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-two pups were separated into three categories depending upon body mass at weaning: low weaning mass pups (LWM, n=7) weighed < 75kg at weaning, average weaning mass pups (AWM, n=19) were 75 to 140 kg and high weaning mass pups (HWM, n=6) were > 140 kg prior to fasting. All LWM pups departed the rookery after 5 weeks of fasting whereas AWM and HWM pups fasted for 9 to 14 weeks. Plasma HBA and NEFA concentrations increased over the first month of fasting and BUN declined over that same period in all pups. However, LWM pups showed higher HBA levels than larger pups after the first week of fasting ($p<0.05$) but were always <2 mM. NEFA concentrations were 2 times higher in LWM pups than in AWM pups throughout their fast and peaked at 5 weeks (LWM=5.46±1.23mM; AWM=3.28±0.94mM). Circulating BUN levels were only significantly higher in LWM pups during the first week. With the exception of lower BUN in HWM pups after 7 weeks of fasting there were no differences seen in fasting metabolite levels in pups over 75 kg weaning mass. Thus, HWM pups may be capable of sustaining a protein sparing metabolism longer than average pups. In contrast, pups less than 75 kg at weaning showed higher lipid mobilization than average pups and showed the expected peak in ketone bodies (which usually precedes departure from the rookery) earlier in the fast.

9.24

NITROGEN EXCRETION IN HUMMINGBIRDS: AMMONOTELY IN A BIRD? Marion R. Preest and Carol A. Beuchat. San Diego State University, San Diego, CA. 92182

Most nitrogen excreted by birds is in the form of urates. 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 high, and they may not fit the avian pattern of uricotely. We measured forms in which nitrogen was excreted by Anna's hummingbirds (*Calypte anna*) with either high or low rates of water flux. When the rate of urine excretion was low, more than 55% of urinary nitrogen was excreted as urate and less than 30% as ammonia. When urinary water loss was high, more than 50% of urinary nitrogen was excreted as ammonia, but less than 40% as urate. This apparent switch from uricotely to ammonotely when urinary water excretion increased does not necessarily reflect a change in amino acid catabolism, but could be a consequence of the need to minimize urinary electrolyte loss. NH_4^+ can substitute for K^+ in Na^+/K^+ ATPase, 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.

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9.25

RESPIRATORY QUOTIENT (RQ) IN *DROSOPHILA MELANOGASTER* DURING STARVATION AND DESICCATION. Minou Djawdan* 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 of flies 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 O_2 metabolism and CO_2 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 generation of selection for starvation and desiccation resistance. Supported by grant US-PHS AG09970.

9.26

EFFECTS OF BODY SIZE AND THERMAL ACCLIMATION ON PARVALBUMIN CONCENTRATION IN WHITE MUSCLE OF STRIPED BASS. Kenneth J. Rodnick and Bruce D. Sidell, Dept. of Biological Sciences, Idaho State Univ., Pocatello, ID 83209 and Dept. of Zoology, Univ. of Maine, Orono, ME 04469.

Parvalbumin from anaerobic white skeletal muscle of striped bass (*Morone saxatilis*) was characterized and hypotheses tested that parvalbumin concentration varies with body size and thermal acclimation. Two parvalbumin isotypes (molecular weights 8.7 and 10.3 kDa, with pIs of 4.63 and 4.90, respectively) were identified from whole muscle extracts by SDS-PAGE, immunoblotting with a monoclonal antibody specific for parvalbumin, and 2-d PAGE of heat-treated supernatants. Total concentrations of parvalbumin were exceptionally high, ranging from 5.2 to 12.3 mg or 0.55 to 1.29 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 fish (<500 g body weight) having two-fold more parvalbumin than large fish (>2 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 muscles of small fish may contribute to higher tail-beat frequency and faster rate of muscle relaxation than in larger animals. Lower titers 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 (5°C) for 8-10 weeks did not change the concentration of parvalbumin in white muscle. (Supported by NSF grants DCB 88-11209 to B.D.S. and Maine EPSCoR 91-08766).

9.27

THE MECHANICS AND ENERGETICS OF HUMAN HAND-RUNNING. James W. Glasheen* 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.

9.28

THE WORK OF RUNNING: DO TENDONS PULL THEIR WEIGHT? T. J. Roberts, R. L. Marsh, C. I. Buchanan, P. G. Weyand and C. R. Taylor, CFS, Harvard University, Old Causeway Rd, Bedford, MA, 01730 and Dept. of Biology, Northeastern University, Boston, MA 02155.

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 medial 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 J/kg muscle). While muscle forces were as great as 60% 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 R01AR18140 to C.R. Taylor, AR39318 to R. L. Marsh and NSF graduate fellowship to T.J. Roberts.

9.29

PERFORMANCE OF THE TRUNK MUSCLES IN HYLIDS DURING MATING CALLS. M. Sarbadhikary* 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 electrical activity of the trunk muscles (external and internal obliques) in *Hyla versicolor* (gray tree frog). These muscles undergo high frequency cyclical contractions to power vocalization. We measured the cycle frequency, muscle strain (change in length as a fraction of the starting length), and the phase of stimulation. A typical call consists of 14-15 cycles with a cycle frequency of 15-25 Hz. A maximum strain of 22% is achieved over the first few cycles. During subsequent cycles the muscles shorten much less. The cycles of shortening and lengthening are asymmetrical with shortening occupying 70% of the cycle time. EMGs precede the shortening phase of length cycle early in the call, but the phase difference reduces as the call progresses, resulting in a delay in EMGs with respect to shortening. Shortening may begin before activation in the later cycles due to the influence of elastic recoil in the system. Using these *in vivo* measurements of length cycle and activation we examined the muscle's performance during similar high frequency contractions *in vitro*. *In vitro* power output during contractions that replicate the natural cyle is quite high (>40 W/kg of muscle at 15 to 18 Hz). The natural length cycle results in more average power per cycle than sinusoidal shortening at the same frequency.

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9.30

A MISMATCH BETWEEN THE CONTRACTILE CAPACITY OF A CALLING MUSCLE AND NOTE REPETITION RATE DURING CALL PRODUCTION IN GREY TREEFROGS. J. D. McLister*, E. D. Stevens, and J. P. Bogart*. Department of Zoology, University of Guelph, Guelph, Ont., Canada N1G 2W1

The Grey Treefrog complex consists of two morphologically identical sister species that differ only in chromosome number and mating call. *Hyla chrysoscelis* (24 chromosomes) has a note repetition rate (NRR) of 28.3 notes per second (nps) at 15°C, 42.5 nps at 20°C, and 56.8 nps 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 nps at 25°C. The contractile dynamics of one of the calling muscles, the tensor chordarum, were compared between these two species. Despite the two-fold difference in NRR between *H. chrysoscelis* and *H. versicolor* at all three temperatures, the twitch time (the interval between stimulation and 50% relaxation) for the tensor chordarum of *H. chrysoscelis* was only 1.5 times faster than that of *H. versicolor* (46.5 ms at 15°C, 30.5 ms at 20°C, and 21.8 ms at 25°C for *H. chrysoscelis*; 68.6 ms at 15°C, 44.8 ms at 20°C, and 32.4 ms at 25°C for *H. versicolor*). At all three temperatures, when subjected to stimulus frequencies equaling the respective NRR of each species, the tensor chordarum of *H. chrysoscelis* underwent less relaxation (40-46%) between stimuli than the tensor chordarum of H. versicolor (70-83%). Similar comparisons of contractile performance were made for the sartorius but no differences were found between the two species. Therefore, it is unlikely that the differences between the calling muscles of *H. chrysoscelis* and *H. versicolor* are due to ploidy difference. Our data imply that, while incapable of calling at the same NRR as *H. chrysoscelis*, *H. versicolor* is physiologically capable of calling at a faster NRR than it does. As *H. versicolor* evolved from *H. chrysoscelis*, and as the calls of *H. versicolor* diverged from the call of *H. chrysoscelis*, changes in the contractile physiology of the calling muscles appear not to have kept pace with changes in the neurological control of NRR.

9.31

CALCIUM ACTIVATION AND MECHANICAL PROPERTIES OF SUPER-FAST MUSCLE FROM TOADFISH AND RATTLESNAKES. Douglas A. Syme, Lawrence C. Rome, Stephen M. Baylor, Stephen Hollingworth and Stan Lindstedt. Univ. of Pennsylvania, Philadelphia, PA 19104; No Arizona Univ., Flagstaff, AZ 86011; The Marine Biological Lab., Woods Hole, MA 02543

Sound-producing muscles operate at frequencies 10-100X greater than those of most skeletal muscles; toadfish swimbladder (*TSB*) muscle operates at 100-300 Hz at 15°C and rattlesnake tail-shaker (*RTS*) muscle at about 100 Hz at 35°C. The free-calcium transients (measured with furaptra) and twitch-contractions of intact fibers, and the force-pCa relationship of skinned fibers, were measured and compared with toadfish red (*TR*) and white (*TW*) myotomal muscle to asses how the super-fast muscles are adapted to operate at such high frequencies. At 15°C *TSB* and *RTS* muscles could be stimulated at 100 and 20 Hz, respectively, and still maintain complete relaxation between stimuli. At 16°C, the amplitude and half-width of the calcium transient averaged 39 μM and 4.3 ms for *TSB* fibers (N=4), 8 μM and 4.3 ms for *RTS* fibers (N=2), 10 μM and 21 ms for *TW* fibers (N=1), and 7 μM and 110 ms for *TR* fibers (N=3). The force-pCa curves of faster muscles were shifted toward higher [Ca]. *TSB* muscle showed half-maximal activation at a relatively low pCa (high [Ca]) of about 5.3, *TW* at about 5.7, *RTS* at about 5.8 and *TR* at about 6.2. The results suggest that the fast twitch kinetics are due in part to a very brief calcium transient, and in part to a relatively low calcium sensitivity, presumably mediated by a fast off-rate of calcium from troponin.

Supported by NIH, NSF, and MRC of Canada.

9.32

EFFECTS OF EPINEPHRINE, ATRIAL NATRIURETIC PEPTIDE, AND ANGIOTENSIN II ON VASCULAR CAPACITANCE IN TROUT. **Yutong Zhang* and Kenneth R. Olson Dept of Biol. Sci., U. Notre Dame & South Bend Ctr. for Med. Ed., Ind. U. Sch. of Med., U. Notre Dame, Notre Dame, IN 46556.**

The effects of epinephrine (Epi), atrial natriuretic peptide (ANP), and angiotensin II (ANG II) on vascular capacitance were investigated in unanesthetized rainbow trout (*Oncorhynchus mykiss*), by measuring mean circulatory filling pressure (MCFP) at 80, 100, and 120% of whole blood volume. Dorsal aortic (P_{DA}) and sinus venosus (P_{SV}) pressures were measured before and during transient electrical cardiac fibrillation (when P_{SV}=MCFP). Whole-body vascular compliance (C) and unstressed volume (USV) were derived from the capacitance curves. Infusion of EPI (370 ng·kg^{-1}·min^{-1}, n=11) increased MCFP at 100% BV from 3.87±0.34 to 4.68±0.51 mmHg (p<0.05), decreased C from 3.28±0.70 to 2.64±0.56 ml·mmHg^{-1}·kg^{-1} (p<0.05), but did not change USV. ANP infusion (260 ng·kg^{-1}·min^{-1}, n=7) decreased MCFP from 4.29±0.30 to 3.57±0.11 mmHg at 100% BV (p<0.05), but did not change C or USV. Infusion of ANG II (115 ng·kg^{-1}·min^{-1}, n=8) did not affect MCFP, C or USV, even though P_{DA} increased 22%, from 27.9±1.1 to 34.1±2.0 mmHg (p<0.01). These results suggest: 1) EPI actively regulates venous tone and influences vascular compliance in trout, favoring blood volume mobilization; 2) ANP modulates vascular capacitance; 3) ANG II affects the arterial vasculature, and has limited influence in overall vascular capacitance. The significance of USV deserves further investigation.

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9.33

ARGININE VASOTOCIN RELAXATION IN GAR (*LEPISOSTEOUS OSSEOUS*) HEPATIC VEIN *IN VITRO*. **D.J. Conklin, N.W. Mick* & K.R. Olson. Dept. of Biol. Sci., U. Notre Dame & South Bend Ctr. for Med. Ed., Ind. U. Sch. of Med., Notre Dame, IN 46556.**

The effects of arginine vasotocin (AVT) were examined in isolated gar arteries (afferent branchial, ABA; conus arteriosus, CA; ventral aorta, VA) and veins (hepatic, HV; intestinal; renal). AVT (10^{-11}-10^{-7}M) had no effect in CA, produced contraction in ABA and VA and stimulated relaxation in veins. In precontracted hepatic veins, AVT response was dose-dependent, long-lived (>30 min) and 10X greater in magnitude than atrial natriuretic peptide (10^{-8}M) or sodium nitroprusside (10^{-4}M). Neurohypophysial analogs also stimulated relaxation in hepatic vein. AVT was more potent when compared with analogs. EC_{50}s for AVT, arginine vasopressin, desmopressin, oxytocin and isotocin were: $5x10^{-10}$, $3x10^{-9}$, $5x10^{-9}$, $6x10^{-9}$, $1x10^{-8}$M, respectively. Strength of relaxation (as % decrease in tension) of AVT and analogs was similar. AVP-receptor antagonists (V1- or V2-type selectivity) were equally effective inhibitors and minimal agonists (<10% of AVT relaxation). AVT-relaxation was not inhibited by indomethacin, propranolol, L-N^G-monomethylarginine and methylene blue. Endothelium removal partially blocked the relaxation (<20% change) but did not alter sensitivity. Forskolin (10^{-7}M) relaxed precontracted gar veins but did not inhibit further relaxation by AVT ($3x10^{-8}$M). AVT appears to act directly on venous smooth muscle cells via a non-classical AVP-receptor, possibly by increasing [cAMP]. This potent response suggests a physiological role for AVT in control of venous tone in the gar. This work was supported in part by NSF Grant No. IBN 91-05247.

9.34

EXOGENOUSLY-APPLIED AND ENDOGENOUSLY-DERIVED ATRIAL NATRIURETIC PEPTIDE IN THE YELLOWFIN TUNA (*Thunnus albacares*): CARDIOVASCULAR RESPONSES, PLASMA NATRIURETIC PEPTIDE CONCENTRATION AND DISTRIBUTION OF IMMUNOREACTIVE GRANULES. John E. Keen[1], Kathy L. Cousins[2], Anthony P. Farrell[2], Richard W. Brill[3] and Ken R. Olson[4]. [1]Hopkins Marine Station, Stanford University, Pacific Grove, California USA 93950, [2]Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. Canada V5A 1S6, [3]National Marine Fisheries Service, NOAA, Honolulu, Hawaii USA 96822, and [4]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 0.00, 0.01, 0.10, 1.00 and 10.00 μg·kg body weight^{-1}) into the ventral aorta (VA) of spinally-blocked yellowfin tuna decreased 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 compounds (thiorphan, C-ANF) which, in mammals, inhibit removal of ANP from circulation, produced similar cardiovascular responses. Radioimmunoassay 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 tuna heart revealed the presence of immunoreactive particles in the atrium with fewer 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.

9.35

A GLUTAMATERGIC SYNAPSE WITHIN THE TRIGEMINAL NUCLEUS MEDIATES THE MAMMALIAN DIVING RESPONSE. Paul F. McCulloch and Nigel H. West. University of Saskatchewan, Saskatoon, Canada, S7N 0W0.

The object of this research was to investigate the role of the trigeminal pathway in the initiation of the mammalian diving response. We simulated diving with nasal water flow plus apnea in anesthetized, paralyzed and artifically ventilated rats. These stimuli produced an immediate bradycardia, heart rate decreasing from 379 ± 8 to 83 ± 22 beats per minute within 2.5s. Infusion of 5 μl 5% lidocaine into the spinal trigeminal subnucleus interpolaris (Sp5I) reversibly eliminated the bradycardia response to nasal water flow plus apnea. To determine if lidocaine blocked synaptic transmission (rather than action potentials along axons of passage), glutamate receptor antagonists D-2-amino-7-phosphonoheptanoic acid (AP-7) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) were sterotaxically infused into Sp5I. Bilateral infusions of 1μl 5 mM AP-7 and DNQX eliminated the bradycardia evoked by nasal water flow plus apnea. The blocking effects of the antagonists were reversible. The bradycardia returned within 3 hrs. We conclude that the afferent pathway initiating the mammalian diving response projects through the trigeminal nucleus, and that conduction in this pathway is mediated by a glutamatergic synapse within the Sp5I.

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9.36

NERVOUS MECHANISMS INVOLVED IN FREE DIVING BRADYCARDIA IN MUSKRATS (ONDATRA ZIBETHICUS). Pierre E. Signore* and David R. Jones. University of British Columbia, Vancouver, BC, V6T 1Z4 Canada.

Most of our knowledge of the nervous control of the diving response comes from anesthetized and forcibly submerged animals. In this series of experiments, we investigated nervous pathways involved in diving bradycardia in free diving muskrats using telemetry. Firstly, we injected various autonomic blockers before free diving sessions. Results showed that free diving bradycardia is parasympathetically mediated, occurs independantly of peripheral vasoconstriction and is controlled by a central catecholamine pathway. Secondly, we made muskrats swim against an increasing water flow. Preliminary results suggest that exercice can override free diving bradycardia through activation of the sympathetic system. Thirdly, we cut in the orbit branches of the trigeminal nerve that innervate the external nasal area and the nasal mucosa (maxillary division and nasociliary branch of the ophthalmic division). Diving heart rate from operated animals showed no difference with control animals. Thus, denervation of most of the nasal area does not affect free diving bradycardia.

9.37

REGIONAL CEREBRAL BLOOD FLOW DURING SIMULATED DIVING IN THE RAT. Glenn P. Ollenberger and Nigel H. West. University of Saskatchewan, Saskatoon, Canada, S7N 0W0.

The objective of this study was to examine the cerebrovascular response to simulated diving in the rat using the cerebral blood flow (CBF) tracer ^{14}C-isopropyliodoamphetamine (IPIA) and quantitative autoradiography. IPIA was infused during simulated diving elicited by nasal water flow plus apnea in anesthetized, paralyzed, artificially ventilated Sprague-Dawley 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 ($ml \cdot min^{-1} \cdot 100g^{-1}$). Rates of blood flow were pseudo-color coded and displayed as a color image. During simulated dives of 50 seconds whole brain blood flow was maintained or elevated without any regional reductions in CBF. Regional changes in blood flow were observed in some discrete brainstem nuclei. If regional cerebral blood flow (rCBF) changes are 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 afferent cardiovascular responses during diving in the central nervous system (CNS). Preliminary results suggest that CBF is maintained and elevated during simulated diving despite the significant associated decrease in cardiac output.

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9.38

GLOMERULAR FILTRATION OF FITC-DEX IN CHICKENS AND RATS. Stephani L.B. Boykin, Richard C. Schaeffer, and Eldon J. Braun 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 its mammalian counterpart. This was accomplished by simultaneously infusing a heterogeneous mixture of fluorescein isothiocyanate-dextrans (FITC-DEX) and ^{14}C-inulin (for GFR determination) into chickens (white leghorn, n=5) and rats (Munich Wistar, n=5). The average molecular weights of the FITC-DEX were 3, 10, 19, 40, 70, and 152 kDa (molecular radii, æ, 15, 23, 30, 43, 58, and 83 Å). FITC-DEX present in ureteral 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-DEX. Results from both species show that the filtration fraction of FITC-DEX approaches zero at an æ of 64Å. While there are no previous data for chickens, it has been reported for mammals (Munich Wistar rats) that the filtration fraction of tritiated dextrans approaches zero at æ 44Å (Chang et al., 1975, Biophys. J.), a value nearly 50% smaller than the present data show. 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 filtration of protein does not occur at the mammalian glomerulus. NSF IBN 9220241; VA merit review grant to RCS.

9.39

Avian renal adaptations to different environments. Giovanni Casotti and Ken C. Richardson* Department of Physiology, 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 and surface area of the kidney and nephron subcomponents was determined using stereology. The length of the loops of Henle and the percentage of looped and loopless nephrons were also determined. The renal ultrastructure was examined 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 subcomponents. Arid zone honeyeaters also had a higher proportion of looped nephrons and significantly longer loops of Henle than did wet zone species. Ultrastructurally, adjacent proximal tubule cells of arid zone species had wide intracellular spaces, filled with interdigitated 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.

9.40

PLASMA OSMOLALITY AND BLOOD VOLUME INFLUENCE CIRCULATING ARGININE VASOTOCIN (AVT) IN THE FLOUNDER (*Platichthys flesus*). Justin M. Warne* and Richard J. Balment*. University of Manchester, M13 9PT. U.K.

Although the neurohypophysial peptide AVT has implied roles in teleost osmoregulation, factors controlling secretion are unclear. The potential influence of acute changes in blood volume and plasma osmolality have been investigated by RIA measurement of plasma AVT in a SW adapted, chronically cannulated preparation of the flounder. Blood volume was lowered by haemorrhage (30% of blood volume), 60 minutes after this procedure circulating AVT was unchanged. In subsequent groups blood volume was expanded by the infusion of 150 mM NaCl (30% of blood volume over 30 minutes), this manipulation resulted in significantly lower 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 B.W.) compared with 150 mM NaCl injected control fish (329.4 ± 3.4 vs 320.4 ± 3.0 mOsmol/kgH_2O). 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.

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9.41

Na^+/K^+-ATPASE IS FUNCTIONALLY COUPLED TO CREATINE KINASE IN GILLS OF A EURYHALINE FISH. Dietmar Kültz and George N. Somero. Oregon State University, Corvallis, Or. 97331-2914

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 agarose electrophoresis and immunotechniques the occurrence of muscle-type, brain-type, and mitochondrial CK is shown in gills, in which muscle-type CK is predominant (59±7.1% of total activity). Evidence for a direct functional coupling between CK and Na^+/K^+-ATPase is given in permeabilized gill cells using the CK inhibitor iodoacetamide which abolishes the competitive channelling of ADP from the external pyruvate kinase reaction to the intrinsic CK reaction in a coupled *in situ* Na^+/K^+-ATPase assay. These results emphasize the significance and the central regulatory role for energy metabolism of a phosphocreatine / CK circuit in situations of high and fluctuating energy demands in fish gills. (DK is supported by the German Academic Exchange Service and GNS by NSF grant IBN 92-06660)

9.42

MODULATION OF BRANCHIAL ION MOVEMENTS BY RAINBOW TROUT AT HIGH pH.
[a]Michael P. Wilkie, [b]Pierre Laurent and [a]Chris M. Wood. [a]McMaster University, Hamilton, CANADA. [b]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 radiotracers ($^{24}Na^+$, $^{36}Cl^-$) to relate changes in unidirectional ion movements across rainbow trout gills during a 72 h pH 9.5 exposure regime, to alterations in internal Na^+ and Cl^- balance and branchial ion transporter number and affinity. High pH exposure resulted in initial 60-70% reductions in Cl^- and Na^+ influx (J^{Cl}_{in} and J^{Na}_{in}, respectively), but only affected outflux (J^{Cl}_{out} and J^{Na}_{out}) to a small degree. J^{Na}_{in} remained depressed, but J^{Cl}_{in} recovered by 3 d of alkaline exposure. The initial reductions in J^{Cl}_{in} and J^{Na}_{in} resulted in respective net Cl^- and Na^+ losses (J^{Cl}_{net} and J^{Na}_{net}) of 150-200 umol$kg^{-1}h^{-1}$. Both J^{Na}_{net} and J^{Cl}_{net} were restored by 72 h. Saturation kinetic analysis revealed transient 50 % reductions in J^{Cl}_{max} and more sustained 70 % decreases in J^{Na}_{max}, which accounted for decreases in ion influx. A complete recovery of J^{Cl}_{max} to pre-exposure levels, ultimately led to re-establishment of J^{Cl}_{in} by 72 h. Internal Cl^- concentrations decreased by about 10 % over the first day of exposure but stabilized thereafter. Despite chronically reduced J^{Na}_{in}, plasma Na^+ concentration was relatively stable. This was due to the gradual development of a counterbalancing reduction in J^{Na}_{out}, which was significant by 72 h. In contrast to the kinetics of Cl^- uptake, a persistently depressed J^{Na}_{max} (by 33 %), and a more pronounced decrease in transporter affinity (4-fold increase in K^{Na}_m) accounted for the persistent reduction in J^{Na}_{in}. Thus, rainbow trout regulate internal ion balance at high pH through differential modification of Cl^- vs. Na^+ transbranchial movements. Currently, electron microscopy is being used to establish if these alterations in ion flux patterns were associated with alterations in trout gill ultrastructure.

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9.43

MAGNESIUM TRANSPORT IN RENAL MEMBRANE VESICLES ISOLATED FROM RAINBOW TROUT. Carolina A. Freire*, Rolf K. H. Kinne, Evamaria Kinne-Saffran, and Klaus W. Beyenbach* Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany, and *Section of Physiology, VRT 826, Cornell University, Ithaca, NY 14853

Renal membrane vesicles were isolated from the kidneys of freshwater rainbow trout (*Oncorhynchus mykiss*) by differential centrifugation with sucrose. The vesicles were enriched with the brush-border membrane (BBM) marker enzymes alkaline phosphatase (5.5-fold) and γ-glutamyl transpeptidase (7-fold). These enzyme enrichments and the sensitivity of ^{28}Mg transport to the voltage generated by Na/D-glucose cotransport suggest that the Mg pathway studied here is located in apical BBM of the proximal tubule. Gradients of Na, H or Cl had no effect on ^{28}Mg transport. Uptake of ^{28}Mg was cis-inhibited by divalent cations (20 mM) according to a selectivity sequence predicted for strong binding sites: Co>Mn>Mg>Ca>Sr>Ba, and was also cis-inhibitited by the trivalent cations La and Gd (0.1 mM). Modulators of Ca channels such as the agonist Bayk 8644 and the inhibitors verapamil and diltiazem had no effects. Moreover, ^{28}Mg transport was cis- and trans-stimulated by SH-group reagents, the organic mercurials PCMB and PCMBS. These results indicate voltage-sensitive Mg transport across the BBM via a Mg-selective channel or carrier. Mg uptake through this transport system would serve the reabsorption of Mg expected in the kidneys of freshwater adapted fish.

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9.44

REGULATION OF K FLUX FLUX PATHWAYS IN TROUT RED CELLS BY PROTEIN PHOSPHORYLATION. Y. Weaver and A.R. Cossins, Department of Environmental and Evolutionary Biology, University of Liverpool, Liverpoo L69 3BX, UK.

Trout red cells display two powerful K flux pathways, the KCl 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 KCl efflux and cell shrinkage, respectively. The protein phosphatase inhibitors, calyculin A and okadaic acid, inhibit the oxygenation-activated cotransporter but not the hypotonically-activated Cl-independent pathway indicating a controlling role of dephosphorylation in KCl cotransporter activation. A series of protein kinase inhibitors has been screened for effects on these pathways. NEM (*N*-ethyl maleimide) caused the slow activation of the KCl cotranporter and the subsequent addition of calyculin A 'clamped' activity at a fixed level. This clamped activity was volume-dependent indicating that the volume sensor was not part of the serine/threonine phosphorylation event. The volume-dependence of the clamped flux was inhibited by other kinase inhibitors. Staurosporine also activated the cotransporter but subsequent addition of calyculin A caused complete inhibition of this flux. Chelerythrine has no effect on the KCl cotransporter but did activate a Cl-independent pathway. These effects are consistent with a model in which cotransporter activity is directly activated by a serine/threonine phosphatase and deactivated by a NEM-sensitive kinase. We suggest that staurosporine acts on a separate kinase which controls the activity of the calyculin A-sensitive phosphatase. This indicates that a complex cascade regulates K flux pathways.

9.45

OSMOCONFORMATION AND IONOREGULATION IN AN AGLOMERULAR EURYHALINE TELEOST. Mark D. Baustian and Klaus W. Beyenbach. Cornell University, Ithaca, NY 14853

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 hematocrit 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 urine changes from predominately SO_4 and Cl salts of Mg, in SW, to predominately 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 [Na] increases 3.7-fold. The volume of spontaneously voided urine increases from 17.7±4.8 to 31.6±8.3 µl/hr/100g. In response to hypoosmotic 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 hypoosmotic to plasma forces the toadfish to lose solute in response to hypoosmotic challenge. To minimize the renal loss of solute the toadfish lowers plasma osmotic pressure.

9.46

ABSENCE OF VOLUME REGULATION IN RESPONSE TO HYPO-OSMOTIC SHOCK IN GILL CELLS OF *MYTILUS*. D.S. Neufeld and S.H. Wright, 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 hypoosmotic 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 ^3H_2O (for total water space) and ^{14}C-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 taurine, 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 taurine, betaine, and K^+ content remained unchanged. Absence of an RVD in tissues from intertidal 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 AGNATHAN, MYXINE GLUTINOSA. Tes Toop*, John A. Donald*, AND David H. Evans. University of Florida, Gainesville, FL. 32611

The Atlantic hagfish, Myxine glutinosa, is a marine osmoconforming agnathan that diverged from other vertebrates over 500 million years ago. Natriuretic peptides (NPs) are involved in salt and water homeostasis in mammals and are implicated in fish osmoregulation. Natriuretic peptide receptors (NPRs) in the myxinoid kidney were examined to establish their relationship with higher vertebrate NPRs. Iodinated atrial and C-type NPs (^{125}I-ANP, ^{125}I-CNP) were used in tissue section autoradiography, competition studies, and guanylate cyclase (GC) assays. Rat atrial and porcine C-type NPs (rANP, pCNP) and rat des[Gln^{18},Ser^{19}, Gly^{20}, Leu^{21} Gly^{22}]ANP-(4-23)-NH_2 (C-ANF, which binds to the mammalian and teleost 'clearance' NPR), were used as competing ligands. ^{125}I-ANP binding sites were observed on the glomeruli, neck segments and archinephric ducts; 4.0 nM rANP competed for 50 % of ^{125}I-ANP sites. ^{125}I-CNP did not bind, although 300 nM pCNP competed for 50 % of ^{125}I-ANP sites. C-ANF failed to compete for ^{125}I-ANP sites. ANP and CNP stimulated cGMP production, but C-ANF did not, demonstrating that the hagfish kidney NPR is GC-linked. This study suggests that GC-linkage of NPRs is an ancient vertebrate characteristic, and that NPRs similar to the higher vertebrate CNP and 'clearance' types are absent in the hagfish kidney; furthermore, the observed NPR appears similar to the mammalian ANP receptor. Supported by NSF Grant DCB 8916413 to DHE, and NIH EHS-P30-ES03828 to the Center for Membrane Toxicology at M.D.I. Biological Laboratory, Maine.

9.48

EXPRESSION OF THE MYOGLOBIN GENE IN ANTARCTIC CHANNICHTHYID ICEFISH. Deena Small-Barry, Bruce D. Sidell and Michael E. Vayda. 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 polyclonal anti-(human)Mb antibodies and a cDNA clone that we isolated from a red-blooded Antarctic Notothenioid fish, *Notothenia coriiceps*, we have demonstrated conclusively that two icefish species, *Chionodraco rastrospinosus* and *Pseudochaenichthys georgianus* express Mb mRNA and polypeptide in the heart ventricle at levels comparable to red-blooded Notothenioid fishes. Another icefish, *Champsocephalus gunnari* exhibits Mb mRNA in ventricular tissue although no detectable Mb polypeptide is observed. The icefish *Chaenocephalus aceratus* lacks Mb mRNA as well as polypeptide, but retains genomic DNA restriction fragments that hybridize to the Mb cDNA probe. In contrast to temperate zone fishes, none of the Antarctic Notothenioid fishes examined to date, including the hemoglobin-expressing species *N. coriiceps, Gobionotothen gibberifrons* and *Trematomus newnesi*, express Mb mRNA or polypeptide in aerobic skeletal muscle tissue. This unusual pattern of Mb expression, and independent loss of expression by two distinct mechanisms, has implications regarding the physiology and evolutionary history of these unique organisms. This work was supported by NSF grants DPP 88-19469 and DPP 92-20775 to B.D.S.

9.49

INTRACELLULAR FATTY ACID-BINDING PROTEIN FROM THE ANTARCTIC ICEFISH *Chaenocephalus aceratus*. MAINTENANCE OF BINDING CHARACTERISTICS AT LOW BODY TEMPERATURE.
Richard L. Londraville* and Bruce D. Sidell. Hopkins Marine Station, Stanford University, Pacific Grove, Ca. 93950 and University of Maine, Orono, Me. 04469.

Intracellular 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, yet the role of body temperature in FABP function has not been investigated. We characterized FABP from a cold-bodied animal, and compared its physical and physiological properties with a homologous mammalian FABP. We purified FABP from the aerobic pectoral muscle of *C. aceratus* (CA-FABP) and found that it is remarkably similar to homologous mammalian FABPs. Molecular weight (14936 Da), isoelectric point (5.2-5.3), and intracellular concentration (0.98 mg g^{-1} wet weight) are very similar to corresponding mammalian values. Amino acid sequence from a 51 residue peptide of CA-FABP indicates it is 74% identical to mammalian heart FABP. Binding dissociation constants (Kds) for a variety of fatty acid-ligands range from 1.3-2.7 μM when assayed at a physiological temperature of 0°C, and are not significantly different from values measured for rat heart-FABP at 37°C in parallel assays. However, CA-FABP does have a significantly less hydrophobic binding pocket than rat heart FABP, as measured by relative quantum yield of a fluorescent fatty acid analogue. 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-bodied animals than endotherms. In general, however, the physical and chemical characteristics of FABPs are highly conserved among animals with greatly different evolutionary histories, suggesting that they play a critical role in cell metabolism. Supported by NSF grant # DPP 92-20775 to BDS.

9.50

FLUIDITY, COMPOSITION AND NA^+/K^+-ATPASE ACTIVITY OF CELL MEMBRANES FROM DIVING MAMMALS.
E.E. Williams[1], G.N. Somero[1], J.R. Hazel[2], C.A. Beuchat[3,4] and B.S. Stewart[4]. [1]Oregon St. Univ., Corvallis OR 97331; [2]Arizona St. Univ., Tempe AZ 87287; [3]San Diego State Univ., San Diego, CA 92182; [4]Hubbs-Sea World Research Institute, 2595 Ingraham St., San Diego CA 92109.

Hydrostatic pressure profoundly affects many fundamental physiological processes, yet some pinnipeds routinely dive to great depths. We measured the effect of pressure on the Na^+/K^+-ATPase activity and fluidity of red blood cell membranes of a deep diver, the northern elephant seal (1500m) and a shallow diver, the harbor seal (450m). At surface pressure (0.101MPa), Na^+/K^+-ATPase activities in the two species were similar (elephant seal=71.4 IU; harbor seal=72.8 IU), and both decreased with increased pressure. At 20MPa (=2000m), activity was reduced by 67% of its value at 0.101 MPa in the harbor seal. However enzyme activity in the elephant seal was much less pressure sensitive, declining by only 13%. There were also differences in membrane fatty acyl content in the two species. Amounts of 22:5 were 4-fold higher in elephant seal membranes, and their cholesterol/phospholipid ratio was lower (0.205 vs 0.264), but not significantly so. Membrane anisotropy (inversely related to fluidity) was also measured in red blood cell fragments from elephant and harbor seals, as well as from fur seals (which dive to 230m), dog, horse and cow. All species exhibited a positive relationship between anisotropy and pressure. Anisotropy at 0.101MPA correlated positively with dive depth, but differences in pressure sensitivity (change in anisotropy/change in pressure) did not. The low sensitivity of Na^+/K^+-ATPase activity to pressure in the elephant seal appears to be distinct from the pressure sensitivity of membrane anisotropy and thus may reflect divergence in enzyme sequence or differences in membrane lipid composition influencing overall order but not pressure sensitivity. Supported by NSF Grants IBN 92-06660 to GNS, IBN 92-05234 to JRH and IBN-93-07024 to CAB.

9.51

INCONGRUENCE OF METABOLISM AND ENZYME CONCENTRATION: PATTERNS OF CARDIAC GLYCOLYTIC FLUX DO NOT REFLECT PATTERNS OF VARIATION IN GLYCOLYTIC ENZYME CONCENTRATIONS
Valerie A. Pierce* and Douglas L. Crawford* University of Chicago, Chicago IL 60637

Previously we have demonstrated fixed differences between two populations of the teleost fish, *Fundulus heteroclitus*, in the cardiac concentrations of two glycolytic enzymes, phosphoglucoisomerase and aldolase. In addition, we found that the concentrations of two other glycolytic enzymes, phosphoglyceromutase and enolase, varied with acclimation temperature in these populations. We have measured the resting glycolytic rate in these populations in order to begin 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 two temperatures, 10°C and 20°C. Glycolytic flux was determined by placing minced hearts in flasks containing 5mM glucose Ringer's solution spiked with 2 μCi/ml ^{3}H-5C-glucose, and incubating for 2 hours with shaking at the respective acclimation temperatures. After the incubation period, the media was sampled and counted in a liquid scintillation counter to determine ^3H_2O production. Since the pentose shunt is negligible in these hearts, glycolytic flux can be calculated from the amount of tritiated water produced. We found no differences in resting flux rate between populations at either temperature ($p>0.25$ in both cases). Thus we find that variation in enzyme concentrations is not reflected in the resting cardiac glycolytic rate. However, we cannot rule out the possibility that the populational differences in enzyme concentration may correlate with glycolytic flux under other conditions, such as anoxia or during exercise.

This work was supported by NSF OCE-9116016 grant to DLC.

9.52

SEASONAL CHANGES IN LEVELS OF UBIQUITIN CONJUGATES AND HSP 70 IN INTERTIDAL MUSSELS.
Gretchen E. Hofmann 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 trossulus*. 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 indices of environmentally induced protein damage. Both of these biochemical parameters are appropriate choices for indicators of protein damage. Ubiquitinated proteins are irreversibly damaged and are degraded by intracellular proteases; stress proteins are heat-inducible and known to re-fold denatured proteins. Gill tissue samples were collected and mussel body temperatures were recorded in the field during summer and winter tidal cycles. Quantities of Ub conjugates and levels of hsp70 were determined using dot blot immunochemical assays and western blots, respectively. The results showed that significant differences in the two biochemical indicators correlated with a 20°C difference in mussel body temperatures. In gill collected from summer-acclimatized mussels, the Ub conjugate levels were 5-6 times higher than those measured in winter-acclimatized mussels. Similarly, the relative quantities of hsp70 isoforms were higher in summer- versus winter- acclimatized mussels. The seasonal differences in the two biochemical indicators of protein denaturation 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 Biotechnology Research Postdoctoral Fellowship to GEH and NSF grant IBN 92-06660 to GNS.

9.53

KINETICS OF GOBY LACTATE DEHYDROGENASES IN RELATION TO ENVIRONMENTAL TEMPERATURE.
Peter A. Fields , George N. Somero and Jeffrey B. Graham. Scripps Instit. Oceanography, UCSD, La Jolla, Ca. 92093

Lactate dehydrogenase (LDH) kinetics of four species of gobies in the genera *Gillichthys* and *Coryphopterus* were studied across a broad range of temperatures to determine how the enzyme has adapted to different environmental temperatures. Because LDH is important in anaerobic metabolism, it should be under strong adaptive pressure 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. mirabilis, G. seta*). Michaelis-Menten constants (Km's) of Pyruvate for LDH from each species were determined at 5 degree intervals from 10 - 40 C. The species which occur in the most thermally stable environments -- *C. nicholsi* and *C. personatus* -- show the greatest changes in LDH Km with temperature (0.11-0.48 and 0.06-0.44 mM Pyruvate, respectively). In comparison, *G. seta*, which experiences annual temperature fluctuations from 9 C to 41 C, showed little change in its LDH Km across the temperature range examined (0.11-0.23 mM Pyruvate). *Gillichthys mirabilis*, which occurs in the same geographic area as *G. seta*, shows a stenothermal pattern of LDH Km in relation to temperature, reminiscent of the *Coryphopterus* LDH Km's (0.13-0.51 mM Pyruvate). We conclude that this occurs because *G.mirabilis*, being an estuarine species, can burrow in the muddy substrate to avoid major temperature fluctuations. *Gillichthys seta*, however, is a tidepool species, and must withstand broad and rapid temperature shifts with little scope for behavioral thermoregulation. This is reflected in the extreme eurythermy of *G. seta* LDH kinetics. This work was supported in part by grants from California Sea Grant, NA89AA-D-SG138, project number 72-C-N, and the National Science Foundation, IBN 92-06660.

9.54

INTERPOPULATIONAL DIFFERENCES IN LACTATE DEHYROGENASE-B TRANSCRIPTION: CHARACTERIZATION OF THE BASAL PROMOTER ELEMENTS.
Jeff A. Segal* and Douglas L. Crawford* University of Chicago, Chicago IL 60637

Northern populations of the teleost fish *Fundulus 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 bound proteins. Here we have begun to functionally define the basal promoter elements using a cell culture system. Three different cell lines (salmon embryonic, salmon cardiac and rat cardiac) have been transfected with constructs containing a luciferase reporter gene driven by various sizes and combinations of either north or south *Ldh-B* regulatory region. This functional analysis indicates that specific segments of 5' regulatory region confer transcriptional activation properties important for basal level transcription. Importantly, none of these regions contain a TATA-box and thus *Ldh-B* basal-level transcription is successfully directed from a TATA-less promoter.

This work was supported by NSF OCE-9116016 grant to DLC.

9.55

ADENOSINE RECEPTOR BLOCKADE INCREASES ANAEROBIC METABOLISM IN HYPOXIC RAINBOW TROUT. Nicholas J. Bernier and David J. Randall. Zoology Dept. University of British Columbia, Vancouver, B.C. Canada, V6T 1Z4.

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 mediate the response of rainbow trout to hypoxia. Treatment of hypoxic rainbow trout (PwO_2 = 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 adrenaline 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.

9.56

THE ROLE OF CORTISOL IN THE ONSET OF UREOGENESIS IN THE GULF TOADFISH, OPSANUS BETA. Todd E. Hopkins,* Chris M. Wood, and Patrick J. Walsh. Univ. Miami, RSMAS, Miami, FL 33156.

Confinement and crowding stresses cause gulf toadfish to switch from ammoniotely to nearly complete ureotely over short periods (e.g. 24 h). We examined the role of a stress hormone cortisol in up-regulating hepatic glutamine synthetase (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: +212%; 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 (+652%) 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 ureogenic response presumably by mobilizing fuels (e.g. glucose, amino acids) and thyroid hormones which alter intermediate metabolism. Supported by NSERC and NSF (IBN 9118819).

9.57

SUPPRESSION OF THE UBIQUITIN-MEDIATED PROTEOLYTIC PATHWAY IN QUIESCENT ARTEMIA EMBRYOS.
Thomas J. Anchordoguy* and Steven C. 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 provide evidence that the reduction in adenylate energy status and the associated intracellular acidosis occurring during anoxia-induced quiescence combine to inhibit,directly or indirectly, the initial step in the ubiquitin-mediated proteolytic pathway in embryos of the brine shrimp Artemia franciscana. The levels of ubiquitin-conjugated proteins drop to 37% of control (aerobic) values during the first hour of anoxia, and reach 7% in 24 h. ATP falls to 5% of control values under anoxia and AMP rises reciprocally. This energy limitation is accompanied by a simultaneous depression of intracellular pH (pH_i). By comparison, when embryos are subjected to artificial acidosis under aerobic conditions (pH_i drops sharply, but ATP does not change for hours), ubiquitin-conjugated proteins decline to 58% after 1 h. Additional studies on recovery from 24 h anoxia or aerobic 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 ubiquitination is maintained despite the return of adenylates 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 ubiquitination 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-9306652].

BIOMEDICAL APPLICATIONS OF MARINE MAMMAL PHYSIOLOGY

10.1

PLASMA CATECHOLAMINES IN BOTTLENOSE DOLPHIN IN WARM AND COOL WATER. M. Heath, S. Ridgway, M. Malik, J. Thomas and W.G. Miller. Naval Medical Research Institute, Bethesda, MD 20889-5607.

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 -70°C until assayed. The concentration of EPI and NE were measured by HPLC 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±33.2 pg/ml (12 samples), respectively. The levels of plasma NE were 636.0±122.0 and 842.7±238.4 pg/ml, respectively. Thus, both catecholamines were somewhat lower in dolphin acclimated to 25°C than in dolphin acclimated to 15-20°C, 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 1564.1±353.6 pg/ml, and two weeks post-transport, plasma NE were 1227.4±158.7 pg/ml. Twelve weeks post-transport plasma NE levels were 697.8±215.3 pg/ml, having returned to baseline levels. Elevated plasma NE is a hallmark of physiological responses to a low temperatures that disappears with acclimation to the new thermal environment. The less than 2.5-fold increase in plasma NE observed in dolphin transported from 25°C to 20°C water is relatively small compared to the 6-10-fold increases observed in other mammals during severe cold stress. The results demonstrate that measurable increases in plasma NE in dolphin occur even when the transitions are to moderately cooler water. Further studies should examine plasma NE as a potential indicator of magnitude of thermal stress.

10.2

EFFECTS OF pH AND TEMPERATURE ON THE OXYGEN BINDING PROPERTIES OF THE BLOOD OF NORTHERN ELEPHANT SEALS (Mirounga angustirostris). P.H. Thorson[1], L.N. Starke[2], and B.S. Stewart[1]. [1]Hubbs-Sea World Research Institute, 2595 Ingraham St., San Diego, CA 92109 and [2] Scholander Hall, Scripps Institution of Oceanography, La Jolla, CA 92093.

Dives of northern elephant seals (Mirounga angustirostris) commonly last for 20 -30 min and occasionally for as long as 2 hrs. Oxygen binding properties of the blood may change during these dives as a result of changes in blood pH and temperature, thereby affecting dive performance. We examined the effect of pH (7.2 vs. 7.4); temperature (33 vs. 37 °C); and seal's age on the affinity of hemoglobin for oxygen in three nursing pups, (ca. 3 days old), two weaned pups (2-3 months old), and five adult northern elephant seals. At normal pH (7.4) and temperature (37 °C) P_{50} was 19.5 for nursing pups, 27.0 for weaned pups, and 31.5 for adults. When pH was decreased to 7.2 the oxygen-hemoglobin (O_2-Hb) dissociation curve was shifted to the right and P_{50} increased by 28-45% in weaned and nursing pups and 10% in adults. Conversely, at 33 °C and pH 7.4 the O_2-Hb curve was shifted to the left and P_{50} decreased by 8-19 % in pups and 32% in adults. At pH 7.2 and 33 °C there was little change in the curve from normal pH and temperature as the combined effects offset each other. The P_{50} values for adult elephant seals are higher than those reported for other phocid seals. That lower affinity of hemoglobin for oxygen facilitates unloading of oxygen to tissues at relatively low PO_2 levels, which is probably very important during long dives. Low temperature reverses that effect slightly but that may not be important if metabolism is reduced, thereby reducing the need for oxygen in most tissues.

10.3

CARDIAC LACTATE DEHYDROGENASE ACTIVITY IN MARINE AND TERRESTRIAL MAMMALS; REACTION TO PRESSURE

Rivera, P.M.* and Castellini, M.A.
Institute of Marine Science, University of Alaska, Fairbanks, AK 99775-7220

Many marine mammal species are capable of both long duration and extremely deep dives. While most dives are probably aerobic, the tissues and organs of marine mammals are certainly capable of withstanding occasional periods of hypoxia and ischemia. During these periods, production and consumption of lactic acid by lactate dehydrogenase (LDH) is critical to tissue homeostasis. We have examined differences between LDH activity in the heart tissue of 3 marine and 4 terrestrial mammal species. LDH activity was measured spectrophotometrically at room pressure (1 ATM) and at 136 ATM (equivalent to a 1360 m dive). We calculated affinity constants (Kms) of LDH for lactate. Results indicate that maximum LDH activity is higher in marine mammal heart tissue than in terrestrial mammals at 1 ATM (271 ± 35.1 and 145.4 ± 20.1 Units/gm tissue, ± S.D., p=0.0018) and at 136 ATM (273.8 ± 36.3 and 148.8 ± 17.3 Units/gm tissue, p=0.0016). The LDH Kms for lactate were higher in marine mammals (Km = 7.1 ± 0.65) than in terrestrial mammals (Km = 4.0 ± 0.55) at room pressure (p=0.004). At 136 ATM, the km values for both groups decreased by a similar amount (21-23%). Interestingly, the kinetic properties of cardiac LDH in marine mammals at depth are identical to that of terrestrial mammals at room pressure. We conclude that regardless of species, the kinetic properties of LDH react similarly to pressure, but that marine mammal cardiac LDH may be adapted so that at pressure the animal faces no different biochemical conditions than a terrestrial mammal at the surface under normal pressure.

ADVANCES IN REPTILIAN AND AMPHIBIAN OSMOREGULATION

11.2

CONTRIBUTION OF THE TOAD URINARY BLADDER TO WATER RECYCLING DURING ACCLIMATION TO NACL SOLUTIONS. Shlomo Shpun and Uri Katz, Dept. of Biology, Technion, Haifa, 32000 Israel.

Water uptake in the toad *Bufo viridis* and other amphibia is confined to the skin. Therefore, an equal amount of urine must be produced by the kidneys at steady state conditions. In this study, we compared the osmotic permeability (P_{os}) and water uptake across the skin *in vivo* (Katz 1987) to calculated values from ureteral urine production. Toads were acclimated to tap water (control), saline (230 mOsm Kg^{-1} H_2O NaCl) and 500 mOsm Kg^{-1} H_2O NaCl. Urine was collected concomitantly through a ureteral catheter and from the urinary bladder. The results show that in the tap water acclimated toads urine production equals the water uptake across the skin, and the calculated P_{os} ($6.77 \pm 1.38\ 10^{-4}$ cm/s) correspond to the *in vivo* measurements ($6.28 \pm 2.04\ 10^{-4}$ cm/s). The ureteral urine osmolality and composition did not differ from that of the urinary bladder urine. In the saline acclimated toads, calculated P_{os} ($9.23 \pm 2.37\ 10^{-4}$ cm/s) did not differ from the *in vivo* measurements ($13.9 \pm 2.91\ 10^{-4}$ cm/s), while urinary bladder osmolality (128 ± 6.1) increased over that of the ureteral urine 96 ± 10.2 mOsm Kg^{-1} H_2O. In the hyperosmotically acclimated toads, the *in vivo* measured P_{os} was $10.8 \pm 5.12\ 10^{-4}$ cm/s) while the calculated one was unexpectedly higher ($27.8 \pm 7.1\ 10^{-4}$ cm/s). The urine osmolality increased from 412 ± 19 in the ureter to 483 ± 10.4 mOsm Kg^{-1} H_2O in the urinary bladder. Significant changes were found in the composition of the urinary bladder urine compared to the ureteral urine. While sodium, chloride and potassium concentrations increased, urea concentration decreased. The results suggest that the urinary bladder contributes importantly to water recycling in the hyperosmotic acclimated toads while in the control and saline acclimated toads the urinary bladder is serving as a water reservoir. (Ref. J. Physiol. (Paris) 82:183-187).

11.3

EXTRACELLULAR Ca^{2+} EFFECT ON BASOLATERAL TETRAETHYLAMMONIUM (TEA) TRANSPORT IN ISOLATED SNAKE RENAL PROXIMAL TUBULES. Y. K. Kim and W. H. Dantzler. Dept. of Physiol., Col. of Med., Univ. of Ariz., Tucson, AZ 85724.

Ca^{2+} is an important determinant of a variety of cell functions. We have examined the effect of the removal of extracellular Ca^{2+} on basolateral TEA uptake and efflux in isolated snake renal proximal tubules (25° C; pH 7.4). 0mM $[Ca^{2+}]o$, produced by adding 0.2mM EGTA to 0mM Ca^{2+} Ringer-solution, increased the 2-min uptake of [^{3}H]TEA by about 18%. In addition, the efflux coefficient for [^{3}H]TEA was increased by 44% (12.79±1.47 vs. 8.89±1.07 nm/sec 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 0mM $[Ca^{2+}]o$. However, our previous data show that depolarization reduced TEA uptake and had no effect on TEA efflux. Intracelluar acidification with 0mM $[Ca^{2+}]o$ might have accelerated TEA uptake via enhanced H^+/TEA countertransport. However, this process would not enhance TEA efflux with 0mM $[Ca^{2+}]o$. Moreover, during 20mM NH_4Cl pulse experiments, intracellular acidification (6.69±0.04 vs. 7.29±0.06 pH U for control) did not increase TEA uptake (24.17±2.53 vs. 21.59±2.27 fmol/min/nl, $p>0.05$) whereas intracellular alkalinization (7.77±0.07 vs. 7.29±0.06 pH U) did increase TEA uptake by 24% (18.99±3.42 vs. 15.34±2.63 fmol/min/nl, $p<0.05$). During acidification with NH_4Cl pulse, 0mM $[Ca^{2+}]o$ did not have an effect on TEA uptake (35.10±7.94 vs. 32.31±5.39 fmol/min/nl for control, $p>0.05$). Thus, 0mM $[Ca^{2+}]o$ 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 DCB 9001985).

11.4

IONIC COMPOSITION OF CHICK EMBRYO AIRWAY LIQUID RESEMBLES AMNIOTIC FLUID. Rob McCullough*, Amy Montgomery* and Thomas A. Davis. Department of Biology, Loras College, Dubuque, IA 52004-0178.

Sheep fetal lung liquid has higher chloride ion concentration and total osmolarity compared to amniotic fluid. Previous studies have shown that most mammalian fetal lungs use 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 osmolarity and Cl ion concentration.

	CHICK AIRWAY FLUID		CHICK AMNIOTIC FLUID	
DAY	[Cl^-]	Tot Osm	[Cl^-]	Tot Osm
14	NA	NA	114.7 +/- 9.9	248.0 +/- 8.0
16	116.0 +/- 7.8	254.5 +/- 9.8	115.4 +/- 7.9	247.7 +/- 3.8
18	115.0 +/- 10.2	241.0 +/- 9.3	114.2 +/- 16.9	242.0 +/- 13.3
Sheep	157.0 +/- 4.1	294.0 +/- 2.0	87.5 +/- 5.0	265.0 +/- 2.0

These results show that the [Cl^-] and total osmolarity of amniotic fluid and airway fluid are not significantly different which leads to the hypothesis that amniotic fluid is inhaled during incubation and may be important in expansion and development of the avian air sacs and lungs. This research was supported by a grant to TAD from the Iowa Academy of Science.

11.5

SIGNIFICANCE OF WIPING BEHAVIOR IN THE INDIAN TREE FROG . H. B. Lillywhite, A.K. Mittal, T.K. Garg and N. Agrawal. Banaras Hindu University, Varanasi 221 005, India.

The Indian tree frog, *Polypedates maculatus* (Rhacophoridae), exhibits complex self-wiping behavior which functions to expel both mucus and lipids from cutaneous glands presumed to be homologous with typical anuran mucous glands. Discharge of secretions occurs synchronously at multiple gland openings when the skin is touched, can also be stimulated by epinephrine or isoproterenol, and is inhibited by the B-adrenergic antagonists propranolol and timolol. These and other findings indicate that tactile stimulation of the skin elicits an adrenergic secretomotory reflex. Thus, wiping behavior reflects voluntary control of glandular secretion, as well as a means of spreading secretions over the body surface. The behavior in *Polypedates* evolved independently but is virtually identical to that of certain phyllomedusine tree frogs (Hylidae) which secrete lipids and then wipe themselves to become transiently "waterproof". However, cutaneous secretions of *P. maculatus* provide only moderate resistance to evaporative water loss, which following wiping is about half that of a comparably sized ranid frog or a free water surface. Therefore, wiping behavior is not restricted to "waterproof" species and possibly evolved before skin secretions provided a significant barrier to evaporation. (Supported by a Fulbright Fellowship to HBL.)

12.1

A DYNAMICAL MODEL OF THE PULSATILE SECRETION OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS. B.Z.Liu*, Y.C.Sun* and Y.W.Liu*. Dept. of Physics, Northeast Normal Univ. Changchun, 130024, China.

We propose a comprehensive dynamical model of the secretory system of hypothalamo- pituitary-adrenal axis. The mathematical formulation of it is:

$$\frac{dx_1}{dt} = 1.7\times10^{-6} + \frac{0.007 + 160x_1 + 18000x_1^2}{1 + 480x_3 + 100x_3^2 + 1200x_1 + 800x_1x_3 + 27000x_1^2} - 0.059x_1 \quad (1)$$

$$\frac{dx_2}{dt} = \frac{5.4\times10^{-3} + 3.5x_1 + 11000x_1^2}{1 + 90x_3 + 0.3x_3^2 + 220x_1x_3 + 230x_1 + 21000x_1^2 - 0.052x_2} \quad (2)$$

$$\frac{dx_3}{dt} = 5.6\times10^{-2} + \frac{0.040x_1 + 160x_1^2 + 0.20x_2 + 840x_2^2}{1 + 0.040x_1 + 410x_1^2 + 0.23x_2 + 180x_2^2 + 170x_1x_2} + 0.0295x_4 \quad (3)$$

$$\frac{dx_4}{dt} = 0.496x_3 - 0.0296x_4 \quad (4)$$

$$\frac{dx_5}{dt} = 0.030x_3 - 0.0097x_5 \quad (5)$$

where x_is denote respectively the plasma concentrations of corticotropin-releasing hormone(CRH), corticotropin(ACTH), free cortisol, CBG-bound cortisol and albumin-bound cortisol. The unit of all x_is is $\mu g/l$, time unit is minute. Some results derived from this model and their corresponding experimental results are given in the following table:

Hormone		CRH	ACTH	free cortisol	CBG-bound cortisol	Alb-bound cortisol	Total cortisol
plasma concentration	Exp.	0.006-0.015 $\mu g/l$	0.015-92				50-200
	Cal.	0.0075	0.041	5.5	120	4.5	131
pulse period (min)	Exp.		80-208				
	Cal.	182	182	182			182
pulse amplitude(ng/l)	Exp.		0.005-0.13				44-63
	Cal.	0.015	0.088	4.3			54.1
production rate	Exp.		5-50 μg/day	9-30 mg/day			9-30 mg/day
	Cal.	5.5μg/day	19	9.3			9.3
MCR (l/day)	Exp.	190-900	2.88×10^3				
	Cal.	5.3×10^2	3.7×10^2	1.5×10^3			
half life	Exp.	11.5-95 min	4.3-25 min		5 day	13 day	50-90 min
	Cal.	12	13	3.6	~5	~15	89

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

12.2

THE SIGNAL CONDITIONING ROLE OF THE GILLS OF AMIA CALVA. Stephen L. Katz. Inst. of Oceanography, La Jolla, CA 92093-0204.

Air-breathing fish, such as Amia calva, have an air exchange organ that increases the oxygen tension of blood that subsequently admixes with venous return on the upstream side of the gills. The animal is believed to regulate air-breathing events at least in part by monitoring the oxygen tension 'signal' in the blood on the arterial (ie. downstream) side of the gills. Thus, it is of interest to determine the effect the gills might have on the variability in blood Po_2 signal that results from airbreaths, which raise the blood Po_2, and interbreath apneas, where the blood Po_2 falls. To examine the influence the gills have on fluctuating venous Po_2, the convection and diffusion processes that occur in the gills were modelled with a numerical, computer simulation. Model parameters were chosen to approximate the situation that exists in Amia calva. The results of this modelling suggest that under physiologically realizable conditions the gills can actually amplify the fluctuations in Po_2 that occur in the venous blood as a result of admixture with return from the air-breathing organ. As a result the blood Po_2 signal to breathe is provided with a higher gain by being on the arterial side, rather than the venous side of the gills. Re-examination of the model suggests that it is the non-linear character of the blood-oxygen affinity curve that imparts the amplification character of the gill exchanger. The consequences of the non-linear shape of the blood oxygen affinity curve are well known, but dynamic modelling has pointed out an unappreciated feature of the cardio-respiratory dynamics of these fish.

12.3

BIOENERGETIC NATURE OF CONSCIOUSNESS.
B. Raymond Fink. University of Washington Med. Center, Seattle, WA 98195

Human consciousness and language depend on profuse use of phosphoryl chemical energy (P~) by the brain. Since P~ is redox in origin, the evolution of consciousness presupposes evolution of suitably plentiful intake of reductant (R) and oxidant (O). The respiratory inflow of O, being quasi-continuous, is rate-limiting but does not necessitate consciousness. Garnering food R, however, and *initiating* the swallowing of R does require consciousness. Conscious initiation of swallow leads to reflex closure of the larynx, the O intake bottleneck. Phylogenetically the primitive air-breathing fish larynx undergoes, -- in amphibian, reptilian, mammalian, and primate vertebrates -- a series of additions to its structure and folding mechanism in step with a matched series of increases in the size and potential complexity of the cerebrum. Each laryngeal addition is made at the pharyngeal end of the larynx, displaces the organ cranio-caudally, and appears to increase the cranio-caudal and maximal anteroposterior and transverse dimensions of the open passage. In monkeys the addition is the elastic subhyoid air sac, which becomes partly solidified in apes and replaced by elastic solid tissue in human, each time with increase in the above-mentioned dimensions of the larynx and brain. The primate phylogenetic developments in the larynx and cerebrum are broadly echoed in the maturation of those structures in the human newborn in its first two years. Parsimony, I suggest, can interpret all these coordinated complexifying sequences as related manifestations of a central unifying morphogenetic force, the flow of redox phosphoryl energy. It starts from the mitochondria in the ovum and finds its latest, most elaborate expression as human consciousness and language in maturing *Homo sapiens sapiens*.

12.4

Phylogenetic patterns of nocturnality and physiological capacity in geckos. Kellar Autumn and Robert J. Full. Dept. of Integrative Biology, Univ. of California, Berkeley 94720.

Nocturnal lizards provide an excellent model system in which to study the effects of a substantial evolutionary shift in environment. Lizards are ancestrally diurnal and the majority of lizard species, genera, and families have remained diurnal. Nocturnality requires activity at body temperatures (T_b) 10-30°C lower than does diurnality. Because ectotherms are profoundly affected by changes in body temperature, this is potentially a major obstacle to nocturnal activity. Optimality theory predicts the coadaptation of thermal optima and activity temperatures. However, we have shown that nocturnality imposes a thermal handicap which constrains growth rate and endurance to submaximal levels. Physiologically, activity at low temperature reduces the maximal rate of oxygen consumption ($\dot{V}_{O_2max}$), and therefore the maximum aerobic speed, which in turn reduces endurance capacity. Frog-eyed geckos are active with an average T_b of 15°C, yet have a thermal optimum for $\dot{V}_{O_2max}$ of 35°C. Data from several gecko species shows that these nocturnal lizards have excellent fuel economy (C_{min} 1/2 to 1/3 that of phylogenetically comparable diurnal lizards of similar mass) which partially offsets the thermal handicap. Yet, C_{min} in a secondarily diurnal gecko, *Rhoptropus bradfieldii*, is high and close to predicted values for ancestrally diurnal lizards. This supports the hypothesis that C_{min} and activity time are evolutionarily concordant.

12.5

How much brain is enough? A. L. Towe and M.D. Mann, U.Wash.Sch.Med., Seattle, WA 98195 and U.Nebr.Med. Cntr, 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) determine its allometric variation with P, L and Z. In both sexes from both sites, their within-species E/P and $E^{1/3}/L$ slopes were similar, but their $E^{1/3}/Z$ slopes were steeper. Their between-sex slopes were nearly equal, and similar to their between-group slopes of 1/5th-1/6th. We argue that the between-groups slope show the ΔE needed for the brain to stay in "proper" proportion when a body changes by ΔP. 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 Ewald, Jack W. Feminella, 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 decreases in snail abundance in streams receiving acidic inputs. However, we know little about the specific physiological effects of acidification, and how essential ions, such as calcium, affect physiological tolerance of snails. *Elimia flava* (Gastropoda:Pleuroceridae), a common snail 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 treatment with 0, 5, or 50 mg Ca/liter. Hemolymph was collected at 0, 3, 6, 12, 24, 48, and 72 hr, and analyzed for pH, HCO_3, Ca, Na, and K. All blood factors differed among environmental pH levels ($P<.0001$) and these differences increased over 72 hr ($P<.005$). Blood HCO_3 and blood pH were the only factors that were affected by environmental Ca ($P<.05$), which suggested that uptake of environmental Ca is necessary to maintain blood acid-base equilibrium. Snail behavior appeared to reflect physiological responses to environmental pH and Ca. Snails at pH 4 were completely inactive over 72 hr, but at pH 5, snail 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 (pH > 4), and (2) responsible for maintenance of normal activity levels.

13.2

IDENTIFICATION AND CHARACTERIZATION OF CRUSTACEAN HYPERGLYCEMIC HORMONE (CHH) FROM TIGER SHRIMP, *PENAEUS MONODON*.

Chung-Yen Lin*, Shu-Hwa Chen* and Ching-Ming Kuo*
Institute of Fisheries Science, National Taiwan University, Taipei 10764, Taiwan, ROC

Neurohormones secreted by medulla terminalis in crustacean eyestalks regulate various physiological phenomena and maintain the homeostasis of internal conditions of the crustacean. The technique of eyestalk ablation employed for induced maturation in Penalid shrimps certainly impacts normal physiological processes other than gonadal development . Efforts were made to examine the effects of eyestalk ablation on glucose metabolism in tiger shrimps, *Penaeus monodon*. Crustacean hyperglycemic hormone (CHH), a very important neurohormone in the sinus gland of the eyestalks, controlled the carbohydrates metabolism, and also played significant roles in reproduction and the molting. The eyestalk neurohormones were extracted with 85 % acetic acid, centrifuged, ultrafiltrated, then purified by HPLC (High Performance Liquid Chromatography) . Two peaks named as CHH I and CHH II showed significant hyperglycemia effects ($P<0.05$). CHH I and CHH II are very similar in amino acid composition and absorance spectrum. The fundamental physiology of CHH functions were further documented. Further studies on amino acid sequences, protein structure and signal transduction will lead to a better understandings of CHH in physiological regulations and its control mechanisms involved.

13.3

FUNCTIONAL CHARACTERIZATION OF ENDOTHELIN RECEPTORS IN AORTIC VASCULAR SMOOTH MUSCLE OF THE SHARK, SQUALUS ACANTHIAS. David H. Evans*, Carrie Cegelis, Mark Gunderson. Mt. Desert Isl. Biol. Lab., Salsbury Cove, ME 04672

Endothelin (ET) is now considered to be the most potent vasoconstrictor known, but the role of ET in fishes is relatively unstudied. ET-1 previously has been shown to contract vascular smooth muscle (VSM) rings from both trout and catfish mesenteric arteries and cardinal veins. The trout ventral aorta contracted in one study, but was refractory in another. We examined the sensitivity of the VSM from the shark 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-Glu-L-Ala-allo-D-Ile-L-Leu-D-Trp-)), specific for the ET_A receptor. ET-1 produced a concentration-dependent contraction ($EC_{50} \approx 15$ 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 constrictory, suggesting that ET_B rather than ET_A 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 suggest strongly that functional ET receptors are indeed present in shark aortic VSM, display high sensitivity to the mammalian peptides, and probably are of the ET_B, rather than ET_A, type, contrary to the situation in mammals. (Supported by NSF IBN-9219122 and IBN-9306997 to DHE as well as EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies at MDIBL)

13.4

REGULATION OF CORTICOSTEROID RECEPTORS IN SALMONID GILLS J. Mark Shrimpton and Stephen D. McCormick. Conte Anadromous Fish Research Center, National Biological Survey, Turners Falls, MA and Department of Biology, University of Massachusetts, Amherst, MA, USA.

We have investigated endocrine regulation of corticosteroid receptors (CR) in gills of salmonids. Coho salmon parr (*Oncorhynchus kisutch*) were treated with 0.5 or 5.0 μg/g recombinant bovine growth hormone (rbGH) dissolved in saline. One week following a single injection or after six weekly injections, gill CR concentration was significantly increased 25% and 40%, respectively, in the higher dose group when compared to controls. Gill Na^+K^+-ATPase activity was also significantly greater in both doses of rbGH injected fish after the six weekly treatments, which conferred a significantly greater saltwater tolerance on the experimental fish. To further characterize regulation of CR in the gills of salmonids, we treated Atlantic salmon pre-smolts (*Salmo salar*) with 0.1 μg/g purified coho salmon GH (sGH), prolactin and somatolactin, 1.0 μg/g ovine GH (oGH), 0.2 μg/g insulin-like growth factor I (IGF-I), 1.6 μg/g triiodothyronine (T_3), and a combination of sGH and T_3 dissolved in physiological saline. Fish were given two injections (day 0 and 4) and sampled on day 8. oGH had a significant effect on gill Na^+K^+-ATPase activity, yet did not affect gill CR concentration or affinity. Na^+K^+-ATPase activity was not significantly altered in the other groups. The combination of sGH and T_3 resulted in a significant increase in CR concentration, whereas either hormone injected alone did not have a significant affect on CR numbers. Other treatments did not alter gill CR. In summary, GH alone increases CR concentration in coho parr, whereas GH and T_3 in combination influence CR concentration in Atlantic pre-smolts. Future experiments will address whether these results represent differences between life stage of experimental animals or species specific differences in CR regulation.

13.5

STORAGE AND RELEASE OF CATECHOLAMINES FROM THE CHROMAFFIN TISSUE OF THE ATLANTIC HAGFISH, *MYXINE GLUTINOSA*. Regina Fritsche', Steve F. Perry and Serge Thomas'. Kristineberg Marine Biological Station, Fiskebackskil, Sweden.

In vivo and *In situ* experiments were performed on the Atlantic hagfish (*Myxine glutinosa*) (i) to characterize the leves of circulating catecholamines during acute stresses, including hypoxia, anoxia or physical disturbance (air-exposure), and (ii) 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 the adrenaline levels were unaffected. Exposure of fish to anoxia or physical disturbance (25 min of air-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 cholinergic receptor agonist carbachol (10^{-5}-10^{-4}M) caused a significant release of catecholamines, yet the likelihood of similar mechanisms operating *in vivo* is doubtful because the hagfish heart is not thought to be innervated. Perfusion with anoxic or acidic saline both failed to elicit catechoalmine release. Further, the elevation of perfusion (input) presure to simulate a rise in venous pressure, as might occur during hypoxia or physical disturbance, was also without effect on release. The addition of pituitary extract to the inflowing saline caused a marked release of catecholamines from the chromaffin tissue. Thus, the mechanism(s) of relese of catecholamines from the heart of hagfish during stress *in vivo* remains unclear, although preliminary experiments suggest the possible involvement of pituitary hormones.

13.6

STRUCTURAL AND FUNCTIONAL RESPONSES OF *ACHETA* MALPIGHIAN TUBULES TO SECRETAGOGUES. J. H. Spring*, B. E. Felgenhauer*, D. W. Duhon and C. L. Bordelon*. University of Southwestern Louisiana, Lafayette, LA 70504.

The Malpighian tubules of the house cricket, *Acheta*, 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 initially 150-200 $pl.mm^{-1}.min^{-1}$ and increased 3-fold following treatment with either cAMP or methanolic 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 morphometry of the basolateral infolds 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 living cells using fluorescence ratio 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 JHS and USDA grant 9301639 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 accomplished by pellets implanted for three weeks; control animals received a placebo pellet. Standard metabolic rate (SMR) was increased significantly (23%) in the T4-treated toads. Hepatic glutamate dehydrogenase 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 (25% 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 71%. T4 increased liver triglyceride content 74%, but decreased hepatic glycogen by 64%. Plasma glucose and triglyceride content were unaffected by treatment, but T4 did elevate total plasma cholesterol significantly (77%). Among the enzymes examined, only hepatic enzymes appeared to be affected by treatment. The enzymes that showed an increase in activity are key regulators of aerobic glycolysis, lipid synthesis, and glycogen catabolism. Increase in lipid synthesis could contribute to the increase in metabolic rate of these toads.

13.8

PLASMA LIPID PROFILES IN JUVENILE ALLIGATORS AFTER REMOVAL OF THE ABDOMINAL FAT BODY.
Valentine A. Lance, Allen R. Place, Michael G. Lynch, Marilyn L. Patton and Ruth M. Elsey. C.R.E.S., San Diego, CA 92112, Louisiana Dept Wildlife and Fisheries, Grand Chenier, LA 70643 and Ctr. Marine Biotech., U. Maryland, Baltimore MD 21202.

The abdominal fat body of the Crocodylia has no homologue in other vertebrates. This organ, situated dextrally below the stomach was originally described as a pancreas or "fatty spleen". Its function remains unknown. Fat bodies were extirpated (FBX) from juvenile alligators and growth monitored for 3 months. Initial body mass 494g ± 18, n = 24 FBX; 490g ± 21, n = 24, sham. Final body mass 955g ± 43 FBX and 1105g ± 69 SH. FBX had no effect on growth. There was no difference between groups in the blood glucose response to an injection of epinephrine. Plasma lipid profiles in SH and FBX alligators were analyzed by TLC-FID following insulin injection. Blood samples were taken prior to and at 6, 12, 24 and 72 hr post-injection. Total plasma triglycerides showed a significant increase at 24 hr in the intact alligators but not in the FBX. In contrast total plasma cholesterol esters decreased in FBX but not in SH. There were no significant differences between the groups in total plasma fatty acids, phopholipids, cholesterol or total lipids.

13.9

THE REPRODUCTIVE CYCLE OF THE DESERT TORTOISE (Gopherus agassizii) IN THE LAS VEGAS, NEVADA AREA.
Lisa A. Morici, Valentine A. Lance, David C. Rostal and Janice S. Grumbles.
C.R.E.S, Zoological Soc. San Diego, San Diego, CA 92112.

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-17B and progesterone were measured in females using radioimmunoassay. Plasma calcium was also measured as an index of vitellogenesis in females. Follicular growth occurred in the fall and was correlated with increased calcium. Mating was observed in the spring and in the fall.Nesting 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. and Sept. (peak levels >250 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

THE PRIMARY FUNCTION OF THE INTERCOSTAL MUSCLES IS LOCOMOTION.
David R. Carrier. Brown University, Providence, RI 02912.

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 pneumotach and activity of the forth, fifth and ninth intercostal muscles in four dogs walking and trotting on a motorized 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 ventilatory activity of the intercostal muscles ceases at the initiation of locomotion in both lizards and mammals. These observations, combined with data from the hypaxial muscles of walking salamanders, indicate that locomotion was the ancestral function of the intercostal muscles. Support: NSF IBN 9258243 and IBN 9306466.

24.2

EFFECTS OF A VARIABLE FLAPPING PATTERN ON RESPIRATORY PATTERN AND MECHANICS IN THE BLACK-BILLED MAGPIE.
D.F. Boggs, K.P. Dial and F.A. Jenkins,Jr. Univ. of Montana, Missoula 59812.

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 see how variable flapping patterns may or may not affect respiratory patterns and how the two may be coordinated, if at all, when the ratio of flaps to breaths varies from 3:1 to 2:1. Dynamic respiratory compliance is reduced in flight to a third or less of the resting value and is less during flapping than during gliding. Respiratory frequency is higher during flapping than during brief glides and breath duration shortens with flap duration. At their preferred slower flight speeds when low amplitude flaps predominate and the ratio is approximately 3:1, their inspiratory (T_I) and expiratory times (T_E) are equal and they coordinate respiratory and wingbeat cycles to insure 2 upstrokes and 1 downstroke during inspiration and 2 downstrokes and 1 upstroke during expiration. This provides two 'assists' and one 'interference' for a net assistance in each phase of the respiratory cycle from the effects of flight muscles on sternal and pelvic movements (Am. Zool.33:141A). In three of five magpies studied during short wind-tunnel flights at various speeds, when the ratio of flaps to breaths shifted to 2:1 T_I was shortened to maintain an advantageous phasic coordination. Upstroke overlaps most of inspiration, downstroke occurs with the transition to expiration, upstroke occurs mid-expiration and downstroke with late expiration which again achieves a net assistance (3 assists and 1 interference) to respiration from flight-induced sternal and pelvic movements. Hence flap to breath ratios alone may not indicate whether phasic coordination can exist since intra-breath timing patterns may change. (Supported by NSF grant #IBN-9206673)

24.3

FRACTAL ANALYSIS OF VENTILATORY CONTROL IN HETEROTHERMIC MAMMALS

Joseph M. Szewczak. Deep Springs College, Deep Springs, CA, *via* Dyer, NV 89010.

During the reduced metabolic state of mammalian heterothermic torpor, the time-scale of ventilatory output slows such that patterns 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 Po2=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 less refined appearance of hypoxic-stimulated breathing may result from attenuated cooperation of control elements in a complex system.

(Supported by NSF IBN-9206441)

24.4

NONLINEAR METHODS FOR THE ANALYSIS OF VENTILATORY CONTROL

Jarrod Millman and Joseph M. Szewczak. Deep Springs College, Deep Springs, CA, *via* Dyer, NV 89010.

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-9206441)

24.5

ACETYLCHOLINE STIMULATES BRAIN BLOOD FLOW RATE IN CRUCIAN CARP THROUGH A NITRIC OXIDE DEPENDENT MECHANISM

Göran E. Nilsson and Patrick Hylland, Vertebrate Physiology and Behaviour Unit, Dept. Limnology, 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 epi-illumination 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 N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine. Also sodium nitroprusside, which decomposes to liberate NO, caused increase in cerebral blood flow rate. By contrast, L-NAME could not block the increase in blood flow caused by anoxia. 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 F. Harrison, Phillip Waclawski*, Scotti Gulinson*, and Katherine Krolikowski* 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 CO_2 elimination by 50% by maintaining P_{CO_2}'s in the air sacs consistently below hemolymph and muscle P_{CO_2}'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 P_{CO_2} and increases in tracheal P_{O_2} relative to normal values depress VR, demonstrating that quiescent locusts regulate internal gas levels rather than maximizing CO_2 excretion or O_2 uptake. Post-exercise 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 JFH.

24.7

INTERACTION BETWEEN CO_2 RESPIRATORY DRIVE AND PULMONARY PRESSURE FEEDBACK IN BULLFROGS. Richard Kinkead and William K. Milsom. Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4

In this study we determed how the different components of the breathing pattern of the frog were affected by 1) 3 levels of respiratory drive (air, 1.7% CO_2 and 3.3% CO_2) 2) with changes in phasic *vs* tonic lung pressure. Experiments were performed on decerebrate, paralyzed, unidirectionally ventilated frogs. "Fictive breathing" measurements, 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 CO_2. 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 CO_2 levels increased breathing frequency, and the increase was progressively greater at greater lung pressures. In frogs ventilated with air and 1.7% CO_2, increasing lung pressure reduced the duration of the non-ventilatory period between episodes but did not affect the number of breaths in each episode. At the highest CO_2 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 CO_2 and lung pressure, the duration of the total breath was only shortened by increasing CO_2. With lung deflation to 0 cm H_2O, the fictive inspiration became equal to or greater than the period of glottal opening. We conclude that pulmonary stretch receptor and chemoreceptor feedback interact to produce the normal coordination of breathing movements and to enhance chemoreptor responsiveness. Supported by NSERC and the Killam trust.

24.8

EFFECT OF TIMING OF THE CO_2-RISE PROFILE ON VENTILATION IN GARTER SNAKES. Robert A. Furilla. 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 CO_2 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 CO_2. Snakes were unidirectionally ventilated with air and CO_2 from a gas mixer connected to a computer. The rate of rise of CO_2 was controlled by the computer which also monitored the snakes ventilation. On inspiration, CO_2 in the lung fell to zero, and at the end of inspiration, CO_2 began to rise according to the computer algorithm. The CO_2-rise profile was split into two phases, an early rise phase (0-2.5% CO_2) and a late rise phase (2.5-5%). When CO_2 rose slowly during both early and late rise phases (214 sec to reach 5% CO_2), breathing frequency was 0.28/min, and when CO_2 rose quickly during both phases (3.3 sec to reach 5% CO_2), breathing frequency was 4.23/min. If one of these rates were used for the first half of the CO_2-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 later 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 CO_2 late in the non-ventilatory period and adjust breathing rhythm, and are probably adjusting rhythm continuously as CO_2 rises. Supported by NSF grant IBN-9316434.

24.9

BLOCKADE OF SMALL CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNELS (sK_{Ca}) AFFECTS FICTIVE GILL AND LUNG VENTILATION IN A TADPOLE, *RANA CATESBEIANA*, BRAINSTEM *IN VITRO*. G.-S. Liao*, R.J. Galante*, A.P. Fishman, L. Kubin* and A.I. Pack. Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA 19104

Because sK_{Ca} channels are involved in regulation of afterhyperpolarization and affect repetitive firing properties of neurons, we studied the effect of apamin, an sK_{Ca} channel blocker, on the fictive respiratory activity recorded from tadpole brainstem. This preparation generates two respiratory rhythms corresponding to lung and gill rhythms of an intact specimen (Galante *et al.*, Soc. Neurosci. Abstr. 18:125, 1992). The neural output changes with the development. Thus, it offers an attractive model with which to study mechanisms underlying rhythmic behaviors and their development. We recorded neural activities from VII and X cranial nerves in brainstems of tadpoles at intermediate developmental stages (VII-XI) while superfusing the preparation with five different concentrations of apamin (0.1-2.5 μM). Apamin increased the amplitude of gill-related activity in a reversible and dose-dependent fashion (at the highest concentration: 290%±86(SD) of the control; n=5). In the time domain, apamin had no effect on the frequency of gill bursts, while it increased the duration of lung bursts in a dose-dependent manner (from 1.5s±0.3 in control, to 2.3s±0.5 at the highest concentration; n=5). Thus, apamin, without disrupting either of the rhythms, had differential effects on gill and lung activities in that only the amplitude of the former and primarily the burst duration of the latter were affected. Because both rhythms are found in most facial motoneurons (Liao *et al*, Soc. Neurosci. Abstr. 19:558, 1993), the effects of apamin must occur at premotoneuronal, including pattern generator, levels. Changes in the role played by sK_{Ca} may underlie some of the developmental changes in the respiratory motor output characteristic of amphibians. (Supported by HL-07713 and HL-49486)

THE PHYSIOLOGY OF BLOOD VOLUME REGULATION

25.1

RED BLOOD CELL, PLASMA, AND EXTRACELLULAR TISSUES SPACES IN RAINBOW TROUT (*ONCORHYNCUS MYKISS*). P.G. Bushnell*, D.J. Conklin, D.W. Duff, K.R. Olson. Biol. Dept. Ind. U. South Bend, IN. 46634 & Ind. U. Sch Med., South Bend Ctr., U. Notre Dame, Notre Dame, IN. 46556.

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 (RBCS, ^{51}Cr-RBC), albumin space (AS, ^{125}I-albumin), and extracellular space (ECS, ^{58}Co-EDTA) were measured in 30 tissues of unanesthetized rainbow trout, after circulation intervals of 0.5,1,2,4,8,16, & 24 hrs. ECS was large (725-438 μL/g wet tissue) in kidney, swim bladder, skin, fins; moderate (312-219 μL/g wet tissue) in skull, spleen, liver, intestine, gills, eye, and caecum; and small (180-53 μL/g wet tissue) in red muscle, fat, 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 gall bladder in <1 hr). Equilibration of (^{51}Cr-RBC) was tissue specific and ranged from 100% equilibration in <0.5 hr (gills, brain, eye, red and white muscle, kidney, spleen, caecum, intestine, swim bladder, 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, swim bladder and gall bladder which had not equilibrated in >24 hrs. A tissue-specific delay in equilibration of ^{51}Cr-RBC (fins, skin) and ^{125}I-albumin (fins, skin, muscle) relative to ^{58}Co-EDTA suggests limitations in RBC perfusion in the former and ^{125}I-albumin extravasation in the latter. These results show that fluid spaces in trout tissues are tissue-specific and exhibit varying circulatory dymamics. Furthermore, the apparent extravasation of ^{125}I-albumin suggest that it is a poor 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*, Tes Toop*, and David H. 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 densest on the efferent edge of the gills. Higher resolution light-microscopic examination of emulsion-coated sections showed specific binding occurred 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-ANF (rat des[Gln^{18}, Ser^{19}, Gly^{20}, Leu^{21}, Gly^{22}]ANP displaced 90% of the 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 affect the amount of receptor protein exposed to the blood. Supported by NSF DCB 8916413; NIHEHS-P30-ESO3828.

25.3

EFFECTS OF GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR I ON SALINITY TOLERANCE AND GILL Na^+,K^+-ATPase IN 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 $\mu g \cdot g^{-1}$) followed 48 hours later by transfer to 34 ppt had significantly lower plasma sodium, osmolarity 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 $\mu g \cdot g^{-1}$). Single injections of GH and IGF-I in fish in fresh water failed to improve salinity tolerance following 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 $\mu g \cdot g^{-1}$) for 4-10 days had greater gill Na^+,K^+-ATPase activity and salinity tolerance than controls. Cortisol implants (50 $\mu g \cdot g^{-1}$) 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 $\mu g \cdot g^{-1}$) alone for 4-10 days 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* LATR. Jonathan C. Wright, Stan Caveney*, Michael J. O'Donnell and Johanna Reichert. 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 whole-body glutamine and glutamate in the terrestrial isopod *Porcellio scaber*. Both amino acids play a significant role in N-sequestration between periods of ammonia excretion. Here, analysis is extended to all major amino acids and to four discrete tissues: hepatopancreas, hindgut, pleopodal endopods, and remaining somatic tissues ('body wall'). The pleopodal endopods are strongly implicated in outward transport of ammonia from the hemolymph and are therefore plausible sites of amino sequestration. Animals were 'ammonia-loaded' for 7 days by exposure to a P_{NH3} of 58 Pa generated by an appropriately buffered solution of $(NH_4)_2SO_4$. Control animals were incubated in a saturated, ammonia-free environment for the same period. Following this, tissues were dissected on ice, transferred to acetone, sonicated and centrifuged prior to AA analysis using reverse-phase HPLC. An acid composite, comprising 23 amino acids, was derivatized concurrently with the tissue samples. Samples were spiked with hydroxyproline and α-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 μmol.AA/g fw, or μmol.AA/g protein. Excepting histidine, AA levels increase following ammonia loading, indicating ammonia sequestration. The main sites of AA accumulation are the body wall and hepatopancreas, the latter showing the largest proportional increase (*ca.* 5-fold) in total AA concentration. The major AA's accumulated are glutamine, glycine and arginine, which account for *ca.* 36%, 21% and 18% of net N-sequestration respectively. Accumulations of glutamate, alanine and proline are also significant. Requisite enzymatic pathways permit these AA's to be metabolized to ammonia when humid conditions favor NH_3 volatilization.

26.2

IS SODIUM UPTAKE COUPLED TO AMMONIA EXCRETION IN THE FRESHWATER-ADAPTED MUMMICHOG (*FUNDULUS HETEROCLITUS*)? Marjorie L. Patrick and Chris M. Wood. Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

There is a current debate whether Na^+ uptake is coupled to the extrusion of a hydrogen ion (H^+) and/or an ammonium ion (NH_4^+) in the gills of freshwater fish. *In vivo* studies utilizing freshwater-adapted mummichog (*Fundulus heteroclitus*) provide evidence for the existence of the former only. Increasing the Na^+ concentration of the water over a freshwater range stimulated and saturated Na^+ uptake (J^{Na}_{in}) while net ammonia excretion (J^{Amm}_{net}) remained unchanged. Both amiloride and low pH exposure significantly inhibited J^{Na}_{in}. While J^{Amm}_{net} was not affected by either treatment, net acid flux became positive ($+J^{H+}$) denoting a net acid (H^+) uptake. Intraperitoneal injection of HCl stimulated net acid loss ($-J^{H+}$) and J^{Amm}_{net} without affecting J^{Na}_{in}. High external ammonia levels temporarily inhibited ammonia excretion but by hour 6 of the exposure, J^{Amm}_{net} had returned to within control values. This recovery was performed without any change in J^{H+} or J^{Na}_{in}. These studies indicate that Na^+ uptake in the mummichog is not coupled to NH_4^+ excretion but is somehow linked to H^+ excretion. (Supported by NSERC).

26.3

PYRIDOXAL PHOSPHATE INHIBITS THE ORGANIC OSMOLYTE CHANNEL IN SKATE ERYTHROCYTES. Leon Goldstein and Erin M. Davis-Amaral*. Brown University, Providence, RI 02912

Recent studies in our laboratory and others indicate that volume-activated (VA) release of organic osmolytes (amino acids, polyols and methylamines) 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 (940 mOsm) or hypotonic (460 mOsm) elasmobranch Ringer with 0.1 mM/1uCi ml^{-1} of 3H-taurine, 3H-myo-inositol or 14C-betaine for 30 min at 15°C. The cells were isolated by centrifugation and assayed for radioactivity. Uptake rates of taurine, betaine and myo-inositol in isotonic media were 3.1±.45 nmols/gRBC·30 min (mean±SE, n=8), 1.22±.18 (n=8) and 0.27±.08, respectively. Hypotonicity stimulated taurine, betaine and inositol uptake 5-15X via the same VA channel. P5P at a concentration of 2mM (~IC_{50} for band 3 activity) inhibited VA taurine, betaine and inositol uptakes 90±2%, 91±2% and 91±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. R. Cossins and Y. 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 antiporters which are activated by adrenergic agonists (β-NHE). Antiporter activity may also be affected by cell volume, pH_i and PO_2 though the interactions between these factors have been precisely defined only in trout red cells. We have compared these interactions in red cells from trout, carp and flounder. Hypertonic shrinkage in a nitrogen atmosphere caused activation of the β-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 flounder β-NHE is shrink-activated and oxygenation-sensitive. By contrast, the carp β-NHE is shrink-activated but comparatively oxygenation-insensitive. Trout β-NHE is shrink-insensitive. Evidently, the antiporter responds to manifold stimuli, the exact responsiveness in any one species being of adaptive significance. The oxygenation block of shrink-induced β-NHE activity can be relieved by calyculin A, a serine/threonine protein phosphatase inhibitor, and this activity can be 'clamped' by *N*-ethyl maleimide acting as a protein kinase inhibitor. The control pathways for volume-activation and oxygenation-sensitivity of the β-NHE both involve control of serine/threonine phosphorylation.
(Supported by Wellcome Trust and N.E.R.C.)

27.2

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

Hypoosmotic swelling of carp red blood cells (RBCs) induced a regulatory volume decrease (RVD), which restored the original cell volume within 140 min in oxygenated RBCs, whereas volume recovery was incomplete in deoxygenated RBCs. The complete RVD in oxygenated RBCs 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. Oxygenation *per se* activated a K^+ efflux from the RBCs. Hypoosmotic cell swelling stimulated an additional K^+ release. The oxygenation-activated and the volume-activated K^+ efflux were both inhibited by DIDS and by replacement of Cl^- by NO_3^-, showing that both types of K^+ release were Cl^--dependent and probably via the same KCl cotransport mechanism. Once activated by oxygenation, the KCl cotransport was further stimulated by cell swelling. Deoxygenation inactivated the oxygenation-induced Cl^--dependent K^+ release, and cell swelling was not a sufficient stimulus to significantly reactivate it. In deoxygenated RBCs, the volume-induced K^+ release was transient and primarily Cl^--independent, 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 RBCs but was important for RVD at both oxygenation degrees. The AA release was about 70% inhibited by DIDS and 50% inhibited by replacement of Cl^- by NO_3^-, suggesting that the AA permeation was partly Cl^--dependent. The data communicate a significant influence of hemoglobin oxygenation on transport mechanisms involved in volume regulation of fish RBCs.

27.3

INTRASPECIFIC PHYSIOLOGICAL VARIABILITY AND HYPOXIA TOLERANCE AMONG INDIVIDUAL DOVER SOLE. Edward M. Goolish*[1], Eric A. Lynn* and Russell D. Vetter* (SPON: G. Kooyman). NOAA, La Jolla, CA 92038; [1]and Scripps Institution of Oceanography, Center for Marine Biotechnology and Biomedicine, U.C.-San Diego, La Jolla, CA 92093

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 (P_{50}), hematocrit, muscle citrate synthase 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 (8 weeks at 0.50 mg O_2/l) survived to a lower oxygen concentration (0.08 mg O_2/l) than normoxic fish (0.22 mg O_2/l). Multiple regression analysis indicated that five variables, most of which are involved in oxygen delivery, were influential in explaining individual variability in hypoxia tolerance; hematocrit, relative gill size, body length, blood oxygen affinity, and ration level. Together 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

SEASONAL VARIATION IN THE ACCLIMATION RESPONSE OF THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*. William L. Seddon* and C. Ladd Prosser. Dept. of Physiology, University of Illinois, Urbana, IL 61801

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 Berenbrink and Christopher R. Bridges. Institut für Zoologie, Lehrstuhl für Tierphysiologie, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany

The red cell pHi and pHe of Atlantic cod was studied in carbon dioxide / bicarbonate buffered media in the absence of catecholamines in February/March. At 10% CO_2 (pHe 6.7) the transmembrane distribution ratio for protons was significantly reduced by 2,4-dinitrophenol, cyanide, low extracellular sodium, low extra- and intracellular chloride and by incubating the red cells in CO_2-free media at similar low pHe. At 1% CO_2 9pHe 7.5) the anion transport inhibitor 4,4-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) lead to a significant decrease of both pHi and the intracellular sodium content. The results suggest that Atlantic cod red blood cells exhibit sodium-dependent chloride/bicarbonate exchange to regulate their pHi under hypercapnic conditions in winter.

BIOCHEMICAL ADAPTATION

28.1

CRYPTOCYANIN AND HEMOCYANIN: FLUCTUATIONS AND FUNCTIONS OF CRAB HEMOLYMPH PROTEINS DURING MOLTING. Nora B. Terwilliger and Clete A. Otoshi. Univ. of Oregon Institute of Marine Biology, Charleston 97420.

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 16S 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 hemocyanins decreased 2.5 fold after the molt, while cryptocyanin levels showed an approximate 5 fold decrease after the molt. Hemolymph levels of cryptocyanin remained low longer than did hemocyanin levels. Since insect hemolymph proteins 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 Allen R. Place*[1] Joseph G. Zurovchak[2] and Edmund W. Stiles[2] [1]Center of Marine Biotechnology, Baltimore, MD 21202. [2]Rutgers University, Piscataway, NJ 08855.

Fall migrant frugivorous birds replenishing fat reserves in eastern temperate deciduous forest 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 mustelina*) 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 phospholipids (3-10%) composed almost entirely (>90%) of oleic (46-68%) linoleic (5-41%) and palmitic (10-29%) acid. In paired-fruit choice trials captive wood thrushes preferred synthetic fruits containing primarily unsaturated (vs. saturated) triacylglycerols. Mean retention time of [^{14}C]-labeled triolein was longer (150 min) in birds when offered in an intermediate-lipid (20% dwt) diet than when offered in either a low-(4%) or high- (50%) lipid diet (~90 min). However, 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 McClelland, Georges Zwingelstein, C. Richard Taylor, and Jean-Michel Weber. University of Ottawa, Ottawa, ON K1N 6N5, Canada; Concord Field Station, Harvard University, Cambridge MA 02138

Individual non-esterified fatty acids (NEFA) concentrations were measured in trained dogs and goats (VO_2max dog/ VO_2max goat=2.2) during treadmill exercise at 40 and 60% VO_2max. 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 (VO_2max). The more aerobic species (dog) had much higher plasma NEFA concentrations for all but one NEFA when compared with a 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 the different plasma NEFA composition in 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 > stearate > linoleate. Linolenate, 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 modified albumin with a higher binding capacity than goat albumin (albumin concentration is the same in the two species (0.54 mM)). 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. Gibellato* 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. The *in vitro* PD across the posterior midgut of molting larvae was 28.5% 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 μmol/min/g, n=6) than in feeding larvae (30.8 ± 1.6 μmol/min/g, n=6). The drop in active ion transport during molting may be due to this diminished aerobic capacity, which normally provides the 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%. 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.

28.5

INTERMEDIARY METABOLISM IN THE HEPATOPANCREAS OF THE TERRESTRIAL SNAIL *Cepaea nemoralis* A CYTOPLASMIC ß-HYDROXYBUTYRATE DEHYDROGENASE J.A. Stuart* and J.S. Ballantyne*, University of Guelph, Guelph, Ontario, Canada, NIG 2W1

The subcellular distributions of enzymes involved in carbohydrate, fatty acid, ketone body and amino acid metabolic pathways 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 ß-hydroxybutyrate dehydrogenase (BHBDH), which interconverts the ketone bodies acetoacetate and ß-hydroxybutyrate, is localized exclusively in the cytoplasm of the hepatopancreas cells. An analogous condition is seen in liver and kidney cells of ruminant mammals. BHBDH activity in all other known instances exists bound to the inner mitochondrial membrane.

28.6

METABOLIC COMPENSATION FOR TEMPERATURE IN WINTER-ACTIVE AND WINTER-QUIESCENT CRAYFISH. Daphne Yee* and Nancy L. Pruitt, Dept. Biol. Colgate Univ, Hamilton, NY 13346

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-quiescent (*Orconectes propinquus*) crayfish. Winter-active crayfish show a significant upward translation in the rate-temperature (R-T) curve of V_{O_2} following acclimation to 5 °C (CA)*vs* ac-climation to 20 °C (WA). Winter-quiescent crayfish show no translation of the R-T curve for V_{O_2}. The V_{O_2} for *Orconectes* are generally more temperature-sensitive in all ranges of experimental temperatures (ave Q_{10} ~ 2.0 for O. p.; Q_{10} ~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-quiescent animals (ave. Q_{10} ~ 1.3) *vs* winter-active animals (ave Q_{10} ~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 L. Martin*. UCHSC, Denver, CO 80262.

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 stragegy, 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 mammalian hibernation. In line with this, we hypothesize that periodic arousals are necessary for the biosynthesis of gene products required for the initiation of hibernation. To address this hypothesis, we are currently employing the technique of differential display to identify liver genes which are induced during interbout arousals. 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 adaptive roles in determining the hibernating phenotype.
Supported by ARO Grant DAAL03-92-G-0019 to S.L.M.

28.8

SEASONAL VARIATION IN GLUCONEOGENESIS IN A MAMMALIAN HIBERNATOR (*Spermophilus 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, gluconeogenesis from endogenous substrates is crucial to replenish carbohydrate supplies required by some tissues. Trigylceride 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. Hepatocytes isolated from *Spermophilus 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 ($1.21 \pm .13$, $1.08 \pm .10$, $0.83 \pm .10$ μmol glucose/g. wet wt./min. ± SEM respectively) and 10mM alanine ($0.46 \pm .07$, $0.35 \pm .07$, $0.33 \pm .06$) did not change with hibernation state, while with 10mM glycerol, rates were twice as high in hibernation and arousal as in summer normothermy ($1.11 \pm .13$, $1.13 \pm .10$, $0.58 \pm .05$, $P=0.003$). 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. Srere 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 normothermy; thus, it is likely that liver function 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 and 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 ARO Grant DAAL03-92-G-0019 to S. L. M.

28.10

OXYGEN AND pH REGULATION OF MITOCHONDRIAL PROTEIN SYNTHESIS. Kurt E. Kwast* and Steven C. Hand. Univ. Colorado, Boulder, CO 80309

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 (pH_i) is thought to promote the arrest of both anabolic 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_i observed under anoxia *in vivo*) suppressed protein synthesis rates by an additional 10%. Taken together, these data indicate a direct role for oxygen depletion in the suppresion of mitochondrial protein synthesis. No differences in translation products were detectable as a function of oxygen tension or pH. Intramitochondrial GTP levels did not change after 1 h of anoxia or as a function of extramitochondrial pH, while ATP levels decreased by up to 48% in response to anoxia. Thus, changes in protein synthesis rate 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'-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF) show that ΔpH 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 *in vivo*. Measurements of the rate of mitochondrial protein synthesis as a function of redox potential, both in the presence and absence of intiation or elongation inhibitors, are currently being conducted.

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28.11

ARGININE KINASE IN MITOCHONDRIA ISOLATED FROM THE POSTERIOR MIDGUT OF THE TOBACCO HORNWORM. M.E. Chamberlin, C.M. Gibellato*, and M.M. White*. Ohio University, Athens, OH 45701.

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 1390.7 ± 70.0 nmol/min/mg protein (n=3). The activity in isolated midgut mitochondria is 540.0 ± 34.4 nmol/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 a 22-fold reduction in the activity of the cytosolic marker enzyme, phosphoglucose isomerase. When suspended in a medium containing 0.1 mM palmitoyl carnitine, 0.05 mM malate, 5.0 mM $MgCl_2$, and 0.6 mM ATP, mitochondria respire at a rate of 15.9 ± 0.9 nmol O_2/min/mg protein (n=5). This rate increases to 82.6 ± 5.2 nmol O_2/min/mg protein upon addition of 5.0 mM arginine (n=5). Arginine, itself, is not oxidized by these mitochondria. The arginine-stimulated respiration is inhibited by 0.01 mM atractyloside, 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.

28.12

CORRELATIONS BETWEEN CUTICULAR LIPID COMPOSITION AND WATER LOSS RATES OF INSECTS: HALF OF THE STORY. Allen Gibbs. University of California, Irvine CA 92717

Rates of evaporative water loss of insects have been correlated with compositional differences in cuticular lipids 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 a function of the composition of the surface lipids. To understand one link in this chain, i.e. how compositional changes affect lipid phase behavior, I examined the physical properties of pure hydrocarbons (HCs) and simple HC mixtures, as a model for more complex natural mixtures. Melting temperatures (T_m) increased with chain length by ~2.5°C per carbon atom. Methylbranching and unsaturation decreased T_m by as much as 50°C, with internal substitutions having the greatest effects. HC mixtures melted at intermediate temperatures, and the phase transitions were broader. Fractionation of cuticular HCs isolated from several species corroborated the model mixture results. The order of decreasing T_m was: saturated > total > branched > unsaturated, and the T_m of total cuticular lipids was close to the weighted average of the melting points of the components.

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28.13

LIPID COMPOSITION OF LARVAE FROM VARIOUS FREEZE-TOLERANT AND FREEZE-SUSCEPTIBLE INSECTS. Nancy L. Pruitt, Cheryl Olmstead*, David Nekoukar* and Olga Naidenko*. Dept. Biol. Colgate Univ, Hamilton, NY 13346

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 larvae from *Eurosta*, as well as from another Dipteran that is freeze-susceptible, the fleshfly, *Sarcophaga crassipalpis*, and the larvae of a Hymenopteran 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 (≥ 50%) in monoenes, mostly 16:1, as opposed to the wasp with ~27% monoenes, mostly 18:1. The wasp was rich in polyunsaturates (PUFA), comprising ~39% of fatty acids, about 2/3rds of which were 18-carbon PUFAs, and 1/3 20-carbon PUFAs. The Dipterans were PUFA-poor, with~15% PUFA in *Eurosta* (all 18 carbons acids), 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. Hazel. 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 membrane cholesterol for activity of an integral membrane protein Na^+/K^+ATPase. We are examining functional roles of cholesterol in BLM 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 native cholesterol/phospholipid ratios correspond to maximal activities of Na^+/K^+ATPase. Using an ethanol delivery system to add cholesterol to membranes we have observed the following: Extent of cholesterol enrichment correlates positively with the ratio of exogenously added cholesterol to membrane protein. Preliminary results indicate that a cholesterol enrichment of only 5% in BLM from cold-acclimated (5°C) fish corresponds to an increase (>10%) in Na^+/K^+ATPase activity when measured at 20°C. This response suggests that when measured at supraphysiological temperatures added cholesterol may offset the fluidizing effect of elevated temperature and restore Na^+/K^+ATPase activity. Supported by NSF IBN 9205234 to J.R.H.

28.15

TEMPERATURE ACCLIMATION ALTERS THE PHASE BEHAVIOR OF TROUT LIVER PLASMA MEMBRANES. Jeffrey R. Hazel and Susan J. McKinley, Department of Zoology, Arizona State University, Tempe, AZ 85287-1501

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-dodecanoyl-2-dimethyl amino- napthalene) and NBD-PE [N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine], were used to study the gel/fluid and H_{II} phase behaviors of the membranes, respectively (laurdan spectra can be resolved into two components originating from gel and fluid phase lipids, while the anisotropy of NBD-PE displays a minimum at the H_{II} phase transition in model membranes). Arrhenius plots of generalized laurdan polarization display distinct discontinuities (believed to reflect the onset of the fluid/gel phase transition) not detected by conventional fluidity probes (*e.g.*, DPH), in membranes of both 5°- and 20°C-acclimated fish. In 5°C-acclimated trout, the transitions ranged from 4-7.5°C (5.8 ± 1.6 °C; Mean ± SEM, n = 4), whereas in 20°C-acclimated trout the transitions ranged from 12-15°C (13 ± 0.63 °C). NBD-PE anisotropies display a distinct minimum at ≈60°C in all membranes, but a distinct secondary minimum at ≈20°C is seen only in membranes from 5°C-acclimated trout. These data document thermal compensation of both the gel/fluid and H_{II} phase boundaries and suggest that the tendency for formation of the H_{II} phase is subject to greater adjustments during thermal acclimation than is the gel/fluid boundary. (Supported by NSF Grant IBN 9205234 to J.R.H.)

28.16

MEMBRANE ADAPTATION TO SOLUTE SYSTEMS J.S. Ballantyne* and H.C. Glemet* Department of Zoology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

The phospholipid 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, the little skate, *Raja erinacea*, and the marine teleost, the winter flounder, *Pleuronectes americanus*. Where possible organisms were maintained at the same 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 gill mitochondria at high salinities consistent with a strategy to counteract the effects of increasing intracellular levels of inorganic ions. Comparison of the membrane 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 high concentrations of urea 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 CALIFORNIANUS* COLLECTED FROM DIFFERENT HEIGHTS IN THE INTERTIDAL ZONE. Deirdre Roberts*, Gretchen E. Hofmann and George N. Somero. Oregon State University, Corvallis, OR 97331-2914.

The objective of this study was to quantify levels of HSP70 stress proteins in the intertidal mussel *Mytilus californianus* during periods of emersion over a spring tide cycle. Gill tissue samples were dissected from mussels at high and low tidal-height sites immediately before exposure to air by the out-going tide and immediately before re-immersion by the in-coming tide in July and February at Strawberry Hill, Oregon. Air, seawater and mussel tissue temperatures were also recorded. HSP70 levels were quantified with western blotting techniques. There were distinct seasonal differences, both in the quantity and banding pattern of HSP70 proteins. The summer samples had higher levels and more HSP70 isoforms than the winter samples. Moreover, in the summer samples there were significant differences in the levels of HSP70 proteins from mussels from the high and low sites. For one HSP70 isoform, the levels in the high site samples, exposed for 8 hours, were five times greater than those from the low site, exposed for 2 hours at low tide. During the same emersion period, mussel tissue temperatures ranged from approximately 9°C to 23°C. The characterization and quantification of stress proteins in field populations in habitats with highly variable temperature conditions may be an important step in our understanding of the functional relevance of HSP70 in relation to elevated temperatures experienced under natural conditions, seasonal and diurnal changes in HSP70 levels, and seasonal thermotolerance.

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28.18

ATP-DEPENDENT PROTEIN DEGRADATION IN EMBRYOS OF THE BRINE SHRIMP, *ARTEMIA FRANCISCANA*. Frank van Breukelen* and Steven C. Hand. Univ. Colorado, Boulder, CO 80309

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 proteolysis is potentially crucial. An *in vitro* assay for proteolysis was developed with *Artemia* lysates, which relied on the liberation of amino acids from endogenous proteins (prelabeled with ^{14}C) or from exogenously added bovine serum albumin (labeled with ^{125}I). Proteolysis was greatest in lysates in which ATP concentration (1 mM) and pH (7.9) resembled levels found in aerobic cysts. Further, this rate was stimulated in the presence of exogenous ubiquitin. When either the ATP concentration or pH was lowered to values more closely representative of quiescence (free ATP, nominally zero; pH 6.7), proteolysis was reduced to 54.3 ± 3.7% (mean ± SE, n=5) of the maximal rate. When both the ATP concentration and pH were lowered simultaneously, proteolytic rate was inhibited by approximately 65%. These results are consistent with an active ubiquitin-dependent pathway for protein degradation in *Artemia* embryos, and with modulation by ATP and pH. Since anoxia-induced quiescence is characterized by dramatic decreases in both intracellular pH and ATP and an increase in AMP, these factors appear to be likely modes of regulation for intracellular proteolysis in *Artemia*. [Supported by NSF grant IBN-9306652]

28.19

CONTROL OF SORBITOL BREAKDOWN AND GLYCOGEN SYNTHESIS IN THE POST-DIAPAUSING GEMMULES OF THE FRESH-WATER SPONGE, *EUNAPIUS FRAGILIS*, DURING GERMINATION. Stephen H. Loomis,[1] Paul E. Fell,[1] and Steven C. Hand,[2] [1]Connecticut College, New London, Ct. 06320 and [2]University of Colorado, Boulder, Co. 80309

Post-diapausing gemmules of the fresh-water sponge *Eunapius fragilis* undergo a marked increase in metabolism during germination. The increased metabolism is at least partly fueled by consumption of sorbitol. Sorbitol levels decline from 6.5 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 12 mg/gfw for the remainder of germination. The breakdown of sorbitol results from an increase in the activity of sorbitol dehydrogenase activity from undetectable levels in dormant gemmules to a maximum of 0.22 μmoles/mg protein/min after 30 hours of exposure to 20°C. Aldose reductase activity remains constant throughout germination. The activity of glycogen synthetase does not change during germination; however, the activity of glucose-6-phosphate-dependent glycogen synthetase is approximately 15 times greater than the activity of glucose-6-phosphate-independent 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 μmoles/min/mg protein to 0.0035 μmoles/min/mg protein during germination. At the same time, however, the percentage of glycogen phosphorylase a decreases from almost 100% to about 80%. This would attenuate the apparent increase in activity. The role of other effectors of glycogen phosphorylase activity remains to be examined.

28.20

CHANGES IN METABOLISM OF THE POST-DIAPAUSING GEMMULES OF THE FRESH-WATER SPONGE, *EUNAPIUS FRAGILIS*, DURING GERMINATION. Steven C. Hand,[1] Stephen H. Loomis,[2] and Paul E. Fell.[2] [1]University of Colorado, Boulder, Co. 80309 and [2]Connecticut College, New London, Ct. 06320

Post-diapausing gemmules of the fresh-water sponge, Eunapius fragilis, remain quiescent when maintained at 5°C. Germination occurs within 48 to 72 hours following warming to 20°C. This study examined changes in the metabolism of the gemmules during germination. Both heat dissipation and oxygen consumption increased steadily during germination and reached 600 to 800% of the original values by the time the sponge emerged from the gemmule. This increase in metabolism could be reversed by placing the gemmules under anaerobic conditions. When aerobic conditions were resumed, the increase in heat dissipation continued. The metabolic status of the gemmules was determined by measuring the adenylate levels and calculating the energy charge, ATP/ADP ratios and the calorimetric to respirometric ratio. Adenylate concentrations remained relatively constant throughout germination with ATP levels at approximately 3.5 to 4.0 nmoles/mg protein, ADP at approximately 0.3 nmoles/mg protein and AMP at approximately 0.5 nmoles/ mg protein. Energy charge remained at about 0.8 throughout germination. The ATP/ADP ratio in quiescent gemmules was relatively low (approximately 7) but more than doubled during germination reaching a maximum of 17.5. Calorimetric to respirometric ratios shifted from approximately 350 to 550 kj/mol O_2 during the early stages of germination and remained high for the rest of the period. These results support a substantial metabolic activation in gemmules following the breakage of diapause, which in these studies was promoted by storage at 4°C for 4 months.

28.21

SCALING OF THE PHOSPHORYLATION RATIO WITH BODY MASS: ARE THERE THERMODYNAMIC LIMITS TO MAMMALIAN BODY SIZE? Geoffrey P. Dobson, and John P. Headrick. 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 ($ATP/ADP.P_i$, 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 -0.25 calculated for the mass specific metabolic rate (or oxygen consumption) of mammals. The Gibbs energy of ATP hydrolysis, ($\Delta G'_{ATP}$), the thermodynamic efficiency of ATP production (energy captured in forming three ATP along the mitochondrial respiratory chain from NADH to 1/2 O_2), mechanical efficiency and the inverse of free cytosolic [ADP] 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. bumble-bee bat, Estrucan shrew and hummingbird) may be set by the thermodynamics of the mitochondrial electron-transport chain.

28.22

MUSCLE GLUCOSE UPTAKE DURING ANOXIA IN WESTERN PAINTED TURTLES(CHRYSEMYS PICTA BELLII). C.L. Watson, T.M. Chen*, W.L. Few*, K. Malvey* and D.C. Jackson. Brown University, Providence, R.I. 02912.

Previous studies have documented that blood glucose concentrations of turtles rise during periods of anoxia. This study measured glucose uptake by skeletal 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/ml ^3H O-methyl glucose, a non-metabolizable analog of glucose added as a radiolabelled tracer. Samples were removed at 10 minute time intervals over 90 minutes. For anoxia studies, turtles were submerged in N_2 equilibrated water (pO_2 < 3 torr) for 14 hours, then dissected and glucose uptake measured in O_2 free conditions. There was no significant difference in glucose uptake between skeletal and cardiac muscle under either normoxic or anoxic conditions. However, glucose uptake was significantly reduced during anoxia in both skeletal and cardiac tissues (96 vs 40 nmol glucose/mg protein/90 min). Considering that metabolic rate of turtles is reduced ten-fold during anoxia, the rate of glucose uptake was signifcantly 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.

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28.23

GLUCOSE UPTAKE IN HINDLIMB MUSCLES OF RUNNING GOATS (*Capra hircus*). T.G. West, J.-M. Weber, C.R. Taylor and P.W. Hochachka. Concord Field Station, Museum of Comparative Zoology, Harvard Univ., Cambridge Ma 02138, USA and Dept. of Zoology, Univ. of British Columbia, Vancouver V6T 1Z4, Canada.

Uptake of ^3H-2-deoxyglucose into semitendinosus (SEMI) and vastus lateralis (VL) of goats provided indicies of glucose utilization (GUI) in hindlimb muscles of resting and running (60% of VO_2-max) animals. At rest, GUI in SEMI was about 3-fold higher than that of the VL. However, exercise resulted in a 60-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 circulatory glucose is a primary component of total carbohydrate oxidation in animals that are not highly areobically adapted.

COMPARATIVE NEUROBIOLOGY

29.1

TRANSNEURONAL INDUCTION OF ATROPHY IN GRASSHOPPER MUSCLES: DEVELOPMENTAL & TAXONOMIC VARIATIONS ON A THEME. AS Clinton* & EA Arbas. ARLDN, U. of Arizona, Tucson AZ 85721.

In grasshoppers, hind-limb autotomy severs the nerve to the leg and, transneuronally, induces atrophy of metathoracic muscles neither damaged nor denervated by autotomy. Extent of atrophy varies with development and across species. Muscles atrophy severely following autotomy at any age in *B. psolus*, but, while robust in juvenile *S. americana* and *M. differentialis*, atrophy diminishes with maturation. 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 muscle fibers atrophy differentially, both within a single muscle and among muscles. In *B. psolus*, 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) Histochemical 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 metathoracic muscles by the common inhibitor motorneuron (CI), including the posterior fibers of m. #120. We compared the extent of atrophy in muscles of this pool and found no strict 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. psolus*, as well as in juvenile and adult *S. americana* and *M. differentialis*. (Supported by NIH T32 NS07363 and NSF IBN 9210394).

29.2

ANOXIA REDUCES N-METHYL-D-ASPARTATE RECEPTOR ACTIVITY IN FRESHWATER TURTLE CEREBRAL CORTEX. Philip E. Bickler and Leslie T. Buck. Dept of Anesthesia, University of California, San Francisco, CA 94143-0542

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 (AJP 235: 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 (fura-2 fluorescence) in brain cortex sheets from *Chrysemys picta*. In brain sheets from normoxic turtles, 50 µM NMDA increased cytosolic calcium ($[Ca^{2+}]_c$) by 497 nM. After 14-18 hours of anoxia, this was reduced to 341 nM ($p<0.05$, unpaired t-test). Anoxia had no significant affect on ATP levels, normoxic - 197.5 and anoxic - 158.4 nmol. ATP/mg protein. Baseline $[Ca^{2+}]_c$ was also unaffected by anoxia, normoxic - 189 and anoxic - 197 nM. These results show that the tissue remains viable throughtout the anoxic period and down regulation of post-synaptic NMDA receptors is involved in the adaptation of turtle brain to anoxia. We speculate that this plays a role in the anoxia-tolerance of the turtle CNS.

Supported by NIH GM52212

29.3

ROLE OF ADENOSINE IN N-METHYL-D-ASPARTATE RECEPTOR MODULATION IN AN ANOXIA TOLERANT TURTLE (*CHRYSEMYS PICTA BELLII*). Leslie T. Buck and Philip E. Bickler. Dept. of Anesthesia, University of California, San Francisco, CA. 94143-0542.

Accumulation of the neuromodulator adenosine (AD) in the anoxia-tolerant turtle brain may play a key role in a protective decrease in excitatory neurotransmission during anoxia. To determine whether AD causes a decrease in NMDA (N-methyl-D-aspartate) receptor activity in the turtle, we measured NMDA-mediated calcium influx in cortical sheets using the Ca^{2+} sensitive dye fura-2. Adenosine decreased Ca^{2+} influx via the NMDA receptor from a control of 287 nM to 103 nM (64%). This effect is mediated via the A_1 receptor since 8 phenyltheophylline (specific A_1 antagonist) effectively blocked the AD effect, and N^6-cyclopentyladenosine (A_1 specific agonist) elicited a similar decrease in the NMDA mediated Ca^{2+} 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 Ca^{2+} currents in turtle cortical slices indicate that part of the adenosine effect is membrane potential dependent. We speculate that adenosine causes hyperpolarization of the postsynaptic membrane and this strengthens the voltage-dependent Mg^{2+} 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.

29.4

DEVELOPMENT OF CO2 SENSITIVITY DURING FORCED METAMORPHOSIS IN THE AXOLOTOL (*Ambystoma mexicanum*). Eugene E. Nattie, Elizabeth Kalyvas*, Nandan Kamath*, and Aihua Li*. Department of Physiology, Dartmouth Medical School, Lebanon, N.H., 03756-0001

We induced metamorphosis in the axolotol, a neotenous gill breathing salamander, by treatment with thyroid hormone (T3). Over a period of 3 weeks the gills regress and air breathing, estimated via "gulps" of air taken at the water surface, increases in frequency. We evaluated the "gulp" response to exposure to 5% CO2 in the water and gas phase as compared to air exposure in two experiments: 1) comparison of untreated controls with gills to treated animals with partial to total gill regression, and 2) a longitudinal evaluation of "gulp" frequency in treated animals during the development of gill regression. In the first experiment, untreated animals (N=9) took 11.0 +/- 4.2 (SEM) "gulps" per hour in water equilibrated with air and 9.2 +/- 2.0 "gulps" per hour in 5% CO2. Treated animals (N= 12) took 11.6 +/- 1 "gulps" per hour in air and 28.6 +/- 6.8 "gulps" per hour in 5% CO2, a significant difference. In the second experiment (N=6), during 21 days of treatment, the "gulp" frequency when exposed to air increased from 6.0 +/- 2.0 to 11.8 +/-2.0 per hour ($P< 0.03$) while the frequency when exposed to 5% CO2 increased from 6.0 +/- 1.0 to 21.3 +/- 2.7 "gulps" per hour ($P< 0.001$ vs control, ANOVA). We conclude that during forced metamorphosis as air breathing increases when gills regress the breathing response to CO2 becomes manifest. We hypothesize that chemoreception is induced either by the T3 treatment or its sequelae. (Supported by HL 28066).

29.5

CORTICAL OSCILLATIONS IN TURTLE CORRELATE WITH SPONTANEOUS VISUAL BEHAVIORS. James C. Prechtl* and Theodore H. Bullock. Neurobiology Unit Scripps Inst. & Dept. Neurosci., UC San Diego, La Jolla, CA. 92093-0201

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 gamma 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 unchanging visual environment.

Epipial arrays of 5 electrodes each were placed symmetrically on left and right dorsal cortices of locally anesthetized, partially restrained pond turtles (*Pseudemys scripta*). Field potentials were band passed between 1-100 Hz and recorded continuously over 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 with one eye leading by tens of milliseconds. All discernible eye movements were associated with cortical spindles although ca. 30% of observed spindles occurred without eye movements. Movement related spindles were bilateral and lasted 1-2 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 20 Hz band and their peak amplitudes were usually between 100 and 300 msec earlier than ipsilateral spindles. Changes or small oscillations in pupil diameter occurred at the completion of most eye movements. Pupillary changes without eye movements also occurred and were associated with similarly lateralized 20 Hz spindles. Eye lid opening evoked similar spindles of large amplitude that lasted 1.2-2.4 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.

29.6

MODULAR MEASURING SYSTEM FOR REGISTRATION OF ELECTROCARDIOGRA-PHIC (ECG, ELECTROMYOGRAPHIC (EMG) AND ELECTROENCEPHALOGRAPHIC (EEG) SIGNALS. Jürgen Staszewski and Günther Warncke, Institute of Neurophysiolgy of the University of Cologne, Robert-Koch-Street 39, D - 50931 Cologne. The registration of biological signals as ECG, EMG and EEG is carried out to a large extent by means of high quality, but very expensive commercial amplifier, filter and monitor systems. Although these systems are often very efficient and can be used immediately, they are developed in most cases for a particular application, i.e., they are non-variable. Therefore, they cannot be converted or further developed. Within the scope of our investigations in hummingbirds (Trochilidae) and zebrafinches (Taeniopygia guttata) we developed a modular measuring system for the registration of biological signals. This system, due to the modular set-up, stands out for its great variability. In all, it consists of eight components: **1: Power Supply:** Current-Noise Filter; Ring-Core Transformer (50W); Rectification Charge Condenser (2x 400µF); **2: Power Supply:** (Stabilization): Charge Condenser (2x10000µF); Stabilization With Current Limitation; (±15V, max 1.2A) Reference Voltage (+10V); **3: Input Switch:** takes up the biological and test signals by help of TTL (Transistor transistor logic) signals; Input 1: (Instrumentation Amplifier); Gain=1; Input Protection (±40V) Input 2: Gain=1; Enable Output 1/2; TTL-Input (Enable); **4: Switch Timer:** Time Selector; TIME 1:LOW =2s - HIGH=58s; TIME 2: LOW=4s - HIGH= 116s; TIME 3: LOW=6s - HIGH=174s; TIME 4: LOW=2s - HIGH=28s; Reset; Software Variable (Microprocessor, Type 8448); **5: Differential Amplifier (PGA 204):** Infinitely Variable Gain From 1-10000; Noise Level ≤10 mV; Off-Set (±500mV) Variable; Input Protection (±40V); Input AC/DC; More Inputs Are Possible; **6: Active Filter:** Infinitely Variable HP (High-Pass Filter) Butterworth, Order n=2, f -3dB (10; 100; 500Hz); LP (Low-Pass Filter) Butterworth, Order n=2, f -3dB (1; 3; 5kHz); Notch Filter. Order n=2, f Notch=50Hz, Bandwidth 25Hz; **7: Active Distributor:** Five Outputs, Digital Gain: 1/10 (Gain 100 Possible), Possible To Programme; Bandwidth (250kHz); **8: Acoustic Monitor:** Optical Control With LED-Line; External Output, Direct Or Comparator Mode, Variable Loudness; All components of this system are interchangeable. Each part of it, especially the amplifier and microprocessor modules are self-contained. The amplifier, the input switch and the active filter can be adapted easily to additional requirements. The cost of such a system is moderate and moreover it can be assembled by an electronic engineer in a short time.

29.7

EFFECT OF SODIUM NITROPRUSSIDE ON ION CHANNELS IN RAT CAROTID BODY GLOMUS CELLS. S.C. Hempleman. Division of Physiology, Dept. of Medicine, University of California, San Diego, La Jolla, CA 92093-0623.

Nitric oxide (NO), a putative neurotransmitter in the carotid body (CB), increases CB glomus cell cyclic GMP levels and inhibits hypoxic stimulation of CB afferent nerve discharge (Wang et al., J. Comp. Physiol. 336(3):419-32, 1993; Prabhakar et al., Brain Res. 625(1):16-22, 1993). In the present study sodium nitroprusside (SNP), a compound which releases NO, increases glomus cell cGMP levels, and also inhibits CB sensory discharge, was used to test for effects of NO on glomus cell ion channel function. Glomus cells were mechanically and enzymatically isolated from CB of 6 day old rat pups, plated on collagen IV coated cover slips, and studied in the whole cell patch clamp configuration using 40 msec step depolarizations from a holding potential of -70mV. Superfusing cells with 100 and 200 μM SNP in a normal external electrolyte gave dosage-dependent increases in the outward (positive) voltage gated transmembrane current. Maximal current increases of 119% and 48% occurred at clamping voltages of -10mV and 0mV, respectively ($n = 5$, $p < 0.05$). The augmented outward current did not inactivate over the 40msec step, and is consistent with increased potassium efflux (a repolarizing influence) or decreased calcium influx (reducing neurotransmitter release), either of which could explain reduced glomus cell excitability by SNP and NO. (Supported by NIH HL 17731).

29.8

DIADENOSINE TETRAPHOSPHATE (Ap_4A) MODULATES BIOGENIC AMINE METABOLISM IN PC12 CELLS AND BRAIN SYNAPTOSOMES. Edward Pivorun*. **Clemson University, Clemson, SC 29634**

Membrane preparations from PC12 cells and synaptosomes exhibit specific receptors for Ap_4A. Adrenal chromaffin cells and neurons of a diverse array of vertebrates co-release Ap_4A, suggesting a neuromodulatory role in the PNS and CNS. The role of extracellular Ap_4A in the nervous system is unknown. HPLC with electrochemical detection monitored the effects of extracellular Ap_4A on dopamine release from and metabolism in PC12 cells. Ap_4A resulted in a selective time and dose dependent increase in extra- and intracellular levels of DOPAC with no effect on the other metabolites. The increase in DOPAC levels suggests that Ap_4A is stimulating monoamine oxidase (MAO) activity. Preincubation with MAO inhibitors prevented the observed increases in DOPAC levels. Preincubation with tyrosine kinase inhibitors also resulted in the inhibition of the induced DOPAC production. PC12 cells and brain synaptosomes preincubated with radioactive dopamine and serotonin, respectively, responded to Ap_4A with dose and time dependent increases in DOPAC and 5-HIAA production. Increased levels were noted in 5 min, with 3 to 4-fold elevations in metabolite levels. This study suggests that Ap_4A is a potent activator of MAO activity and that this purinergic agent may have a neuromodulatory role in the vertebrate nervous system by stimulating biogenic amine metabolism.

39.1

CLONING AND EXPRESSION OF SALMONID CARDIAC TROPONIN C: ROLE IN TEMPERATURE SENSITIVITY OF CARDIAC MYOFIBRILS. C. D. Moyes*, T.J. Borgford, L.LeBlanc, G.F.Tibbits. Simon Fraser Univ., Burnaby, BC, V5A 1S6, Canada

The relationship between 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, troponin C (TnC), in determining these properties. Cardiac TnC has 3 functional Ca^{2+} binding sites. Steady state occupancy of the 2 high affinity sites promotes binding of TnC into the troponin complex. Beat-to-beat binding to the single regulatory Ca^{2+} binding site is responsible for activation of myofibrillar ATPase. Rainbow trout cardiac cTnC was cloned (lambda Zap), sequenced and expressed in a bacterial system (*E. coli* strain QY13) as a fusion protein (cII-TnC). Comparison of salmonid TnC amino acid sequence with those of mammals reveals complete homology in the single regulatory Ca^{2+} binding site. Several differences in primary structure occur in the inactive site I and throughout the high affinity domain. These regions are not directly involved in Ca^{2+} binding at the regulatory site, however they are implicated in Ca^{2+} dependent interactions with TnI. Recombinant salmonid TnC exhibits spectral properties (circular dichroism, intrinsic fluorescence) resembling those of TnC purified from Atlantic salmon ventricle. To facilitate titration with Ca^{2+}, a mutant TnC was produced (FW27-cTnC) by site-directed mutagenesis (single-stranded PCR). Introduction of a tryptophan residue into the non-functional site I results in a protein which increases in fluorescence several fold in response to Ca^{2+} binding at the functional regulatory site II. Ca^{2+} affinity of isolated TnC differs in several important respects (temperature and pH sensitivity) from that of intact myofibrillar preparations (ATPase, isometric tension). These data suggest that the differences in Ca^{2+} sensitivity between species are not due solely Ca^{2+} binding properties of TnC but also depend upon functional interactions between TnC and other troponin proteins. Funded by NSERC (Canada)

39.2

TEMPERATURE SENSITIVITY OF SALMONID MYOCARDIAL Ca^{2+} SENSITIVITY C.Churcotte, C.D.Moyes*, G.F.Tibbits*. Simon Fraser Univ., Burnaby, V5A 1S6, Canada

Mammalian heart demonstrates marked decreases in Ca^{2+} sensitivity and isometric tension in response to decreasing temperature. Poikilothermic vertebrates must maintain myocardial contractility at temperatures that are cardioplegic for mammals. We postulated that interspecies differences in myofibrillar Ca^{2+} sensitivity ($K_{0.5}CA$) or its temperature dependence ($dK_{0.5}CA/dT$) may prevent de-sensitization in salmonid heart at low temperature. As $K_{0.5}CA$ is also pH dependent (approx. 1pCa/pH), the pHi regulatory strategy influences functional Ca^{2+} sensitivity *in vivo*. We compared the influence of temperature and pH changes in Ca^{2+} sensitivities of ventricular myofibrillar ATPase and skinned fiber force generation from rat and rainbow trout. Salmonid myofibrils are inherently more sensitive to Ca^{2+} than are mammals, when assayed under similar conditions (pH 7.0-7.2: 7-21°C). Salmonids exhibit a greater sensitivity to temperature at fixed pH (0.35 pCa/10°C) than do mammals (0.08-0.16pCa/10°C). Although salmonids demonstrate temperature dependent desensitization, the higher inherent Ca^{2+} sensitivity results in similar *functional* Ca^{2+} sensitivities when assayed at their respective physiological temperatures. There is a potential role for intracellular pH regulation in modifying the effects of temperature on Ca^{2+} sensitivity. An α-stat pattern of intracellular pH regulation (-0.014pH/°C) would negate the effects of temperature in mammals. Although salmonids and mammals have a similar pH sensitivity, salmonids exhibit a greater temperature sensitivity. This property, along with the established "less-than-α-stat" pattern of cardiac pHi changes, suggests that pHi regulation *in vivo* would be insufficient to overcome the effects of temperature on Ca^{2+} sensitivity. We conclude that one mechanism by which salmonid heart is able to maintain contractility at low temperatures is through higher Ca^{2+}-sensitivity of the contractile element, compared to mammalian species. Supported by NSERC (Canada).

39.3

IS ONE PERFORMANCE MEASUREMENT ENOUGH? ALTERNATIVES TO U_{crit} PROCEDURES FOR EVALUATING FISH LOCOMOTOR PERFORMANCE. **Jay A. Nelson, Shannon Reidy, Dale Webber and Steve Kerr. Dalhousie Univ. Halifax, N.S., Canada B3H 4J1**

Ever since J.R. Brett introduced the critical swimming speed protocol (U_{crit}) in 1964, it has been the method of choice for investigators wishing to evaluate locomotor performance in fishes. Unfortunately, while the procedure has the advantage of producing a single performance number for each fish, little information is obtained concerning either the aerobic or anaerobic capabilities of a particular animal. Indeed, recent work from R.G. Boutilier's laboratory demonstrates that Atlantic cod (*Gadus morhua*) with identical U_{crit}'s can use substantially different amounts of anaerobic metabolism during their swimming effort. Furthermore, under certain environmental conditions, the U_{crit} test becomes almost entirely a test of aerobic swimming capacity in cod. For these and other reasons, we developed two alternative swimming performance tests designed to evaluate an animal's anaerobic swimming capacity. The first test, which we term "burst performance protocol", U_{burst}, is nothing more than an accelerated U_{crit} protocol whereupon the animals are forced to swim against water accelerating at a rate of 10 cm/s/min until exhausted (about 7 min). Measurements of U_{burst} in cod are repeatable and are correlated to U_{crit}. The second test involved measuring "fast-start performance" with a computerized "drag strip" utilizing laser light detection. Measurements of acceleration and terminal velocity during the fast-starts were repeatable for Atlantic cod, both on a given day and over several months time. The relative advantages and disadvantages of these methods as well as the methodological details of these tests will be presented.

39.4

Environmental Determinants of White Muscle Performance: Lessons From the Deep-Sea. Russell Vetter, N. Chin Lai[1], and Edward Goolish[1]*. NOAA, La Jolla , CA 92038 and [1]Scripps Institution of Oceanography, Center for Marine Biotechnology and Biomedicine, U.C. San Diego, La Jolla, CA 92093.

Oxygen-delivery properties (hematocrit, p50, hemoglobin, heart size), and metabolic-demand properties (white muscle and heart lactate dehydrogenase, malate dehydrogenase, and citrate synthase activities), were measured in fish species that occupy different, narrowly-defined depth ranges, and in confamilial species that ontogenetically migrate from shallow to deep water. Four Scorpaenid rockfish species and four Pleuronectid flatfishes were collected at 100m depth intervals from the continental shelf (100m) down to (1400m) including the Oxygen Minimum Zone (<.5 ml/l O_2 at 600-800m). By using the shallow-water representatives of each family, we were able to examine the environmental effects ofdeep water (darkness, hypoxia and low-food availability), as they were overlain on accepted patterns of metabolic scaling. The shallow-water rockfish, *Sebastes goodei*, and flatfish,*Paralichthys californicus*, showed size-specific increases in LDH and decreases in CS These were in accordance with cost-of-transport arguments. Deeper-living species had progressively more inverse (negative) size-scaling relationships for LDH, and steeper decreases in CS with increasing body size. The ontogenetically migrating flatfish, *Microstomus pacificus*, took on the pattern of the deep-water species when it migrated to deeper water. Laboratory experiments with *Microstomus pacificus* confirmed that muscle performance is strongly influenced by the deep-sea environment. Fish removed from deep water and maintained in the laboratory took on scaling patterns and oxygen delivery properties typical of shallow-water. Long-term experimental hypoxia revealed remarkable interactions between hypoxia and ration such that fish maintained under hypoxia grew as well or better than normoxic controls, but did so by consuming half the ration. (Supported by NOAA and the National Academy of Sciences)

39.5

REVERSIBLE BINDING OF PHOSPHOFRUCTOKINASE TO MYOFIBRILS DURING EXERCISE AND RECOVERY. Hélène Migault * and Mary Sue Lowery. University of San Diego, San Diego, CA 92110.

Phosphofructokinase (PFK) binds to fast-twitch, glycolytic (white) muscle myofibrils during exhaustive exercise in the barred sand bass, Paralabrax nebulifer. In rested fish, 43.1% of white muscle PFK is bound to myofibrils, with bound PFK increasing to 65.9% in exercised fish. Increased PFK binding corresponded to a decrease in muscle pH from 7.26 to 6.56 and an increase in muscle lactate from 11.4 to 31.2 mmoles/g in exercised fish. During the initial phase of recovery, PFK binding decreased as the intracellular muscle pH and lactate returned to resting values. However, PFK binding increased at 9 hours of recovery indicating that factors other than pH may contribute to binding. Total PFK activity doubled after exercise and continued to increase during the first 3 hours of recovery. Both cytoplasmic and myofibril-bound PFK activity increased, with a larger increase seen in bound enzyme. PFK activity returned to resting levels by 9 hours of recovery. PFK responds to decreased intracellular pH by binding to myofibrils, as seen *in vitro*. Contrary to results of *in vitro* kinetic studies where low pH inhibits PFK, PFK activity in exercised muscle increased under acidic conditions. Reversible binding to myofibrils may serve to enhance glycolysis during exercise or protect PFK activity during extended acidosis.

39.6

AEROBIC AND ANAEROBIC CAPACITIES IN LOCOMOTOR MUSCLE OF TUNAS AND ECTOTHERMIC SCOMBRID FISHES. Michael Hansen* and Kathryn Dickson. California State University, Fullerton, CA 92634

Tunas (Family Scombridae) are unique among teleost fishes in using physiological mechanisms to maintain muscle temperatures elevated significantly above ambient water temperature (endothermy). The maximal activity of key enzymes that limit flux through ATP-generating pathways in fish muscle were used as indices of metabolic capacity, and were compared in six tunas and four ectothermic scombrid species. In red myotomal muscle (RM), the activity of citrate synthase (CS), an index of aerobic capacity, did not differ significantly between the endothermic tunas and the ectothermic scombrid species. In white myotomal muscle (WM), on the other hand, tunas had significantly greater activities of both CS and lactate dehydrogenase (LDH), and index of anaerobic capacity, as well as a greater buffering capacity. Thus, tunas and their closest relatives, ectothermic scombrids, have similar RM aerobic capacities, which may be near maximal for locomotor muscle, but only the tunas maintain elevated RM temperatures, which may be as much as 13°C above water temperature in free-swimming fish. By elevating the temperature of a relatively small amount of tissue (RM is 4-13% of body mass in tunas), tunas can significantly increase red muscle ATP production rate, contraction speed, and power output, and thereby may be able to increase sustainable swimming performance. Tuna WM, on the other hand, lacks extensive heat exchangers and has lower and more variable temperatures than RM; its aerobic and anaerobic capacities are greater than those of ectothermic scombrids. The greater WM aerobic capacities suggest that tuna WM is comprised of more than one muscle fiber type, that it contracts aerobically during sustainable swimming, and/or that it participates in processing the lactate generated during anaerobic bursts. The high WM anaerobic capacity probably results in higher burst speeds and/or duration of bursts in tunas.

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. Expected correlations between burst speed and enzymatic activity, muscle contractile properties or morphology among individuals are rarely detected. Typically enzymatic determinants are sought among activities of glycolytic enzymes; this might not be fruitful if glycolysis plays a minor role in energy turnover during the few seconds over which burst speed is measured. Enzymes associated with rates of muscle contraction may be better predictors of burst locomotor speed. In this study I regressed maximal burst speed of Pacific Tree Frog tadpoles on body size, tail size, muscle mass, and the activities of myofibrillar ATPase and calcium ATPase. Maximum burst speeds were measured at 30°C in an aquatic racetrack and scored as the fastest single 15 cm interval. Following race trials body length, tail length, and tail height were recorded. Tail musculature was dissected, weighed and frozen for enzyme assays. Forty three animals were used. Preliminary results show no dependence of burst speed on any morphological measurements except tail height, leaving open the possibility that enzyme activities are more important determinants of speed. This project was supported by Sigma Xi, ASIH, SSAR, and NSF grants IBN 8918054 and IBN 9118346.

39.8

CORTISOL INHIBITS METABOLIC RECOVERY FROM EXHAUSTIVE EXERCISE IN RAINBOW TROUT. C. Louise Milligan*, Antonella Pagnotta and Steve Eros Dept. of Zoology, Univ. of Western Ontario, London, Ont. N6A 5B7 Canada

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 resynthesis. The exercise-induced rise in plasma cortisol was blocked by either treatment with metyrapone (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 successfully 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, metyrapone-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 and glycogen resynthesis was similar to that in control fish. Our working hypothesis is that cortisol exerts its effects through stimulation and/or inhibition of the muscle glycogen phosphorylase/synthase system.

39.9

BIOCHEMICAL INDICATORS OF GROWTH IN THE SNOW CRAB CHIONOECETES OPILIO (O. FABRICIUS). Elise Mayrand*, Helga Guderley and Jean-Denis Dutil*. Dep. Biologie, Université Laval, Québec, Qué., Canada. G1K 7P4.

This study aimed to identify biochemical indicators 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 biochemical variables was first assessed. Mitochondrial enzyme activities were higher in mature 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 postmolt were exposed to different food rations and were collected after 25 and 60 days of experimentation. Muscular tissue growth was highly correlated with the hepatosomatic index and the activities of cytochrome-C oxydase, citrate synthase and lactate dehydrogenase but was independent of phosphofructokinase 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 DFO, Budget Spécial de Recherche de l'Université Laval and NSERC.

39.10

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

The dragonfly *Libellula pulchella* shows a novel developmental pattern of adult myogenesis accompanied by a large-scale change in thermal sensitivity of flight performance. Newly emerged adults (tenerals) 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 ecological/evolutionary significance of this trait. Changes in muscle color (white in tenerals; red in matures) and ultrastructure (hypertrophy of mitochondria in matures) 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 anaerobic end-products (lactate) during continuous muscle activity, and 3) energy stores (glycogen, fat) in *L. pulchella* dragonflies of various ages. LDH activity in nymphs was high (50-60 uM product/g tissue/min), decreased by about half in tenerals, and in matures was almost nonexistent. Citrate synthase activity increased from very low in nymphs to levels in excess of 60 uM product/g tissue/min in matures. However, glycogen content and lactate accumulation were consistently low for all developmental stages, and the rate of fatigue was indistinguishable between teneral and mature muscles. These results indicate that even though tenerals 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 NSF grant IBN-9317969.

39.11

SURFACE-SKIMMING STONEFLIES: DEMONSTRATION OF A POSSIBLE INTERMEDIATE STAGE FOR THE EVOLUTION OF INSECT FLIGHT. James H. Marden and Melissa G. Kramer*. 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 forms for ventilation and swimming, yet the nature of intermediate stages in the evolution of flyers from swimmers remains a mystery. Here we describe a form of non-flying aerodynamic locomotion used by stoneflies, called "surface-skimming", in which thrust 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 of stoneflies as a function of wing area (manipulated by clipping off various amounts of the wings), temperature, and flight muscle ratio (flight muscle mass/total body mass). Surface-skimming velocity improved incrementally with incremental increases in each of these variables. Stoneflies were capable of surface-skimming with wing area reduced by as much as 80% and muscle power output severely restricted (thoracic temperatures as low as 1.5°C). Thus, surface-skimming is a mode of locomotion wherein all components of the flight motor (wings, wing articulations, muscle, and neuromotor patterns) could have simultaneously undergone directional selection for improvement in flapping aerodynamic performance, starting from ancestral aquatic insects with small protowings and low muscle power output. Morphological features of stoneflies that appear to be specific adaptations for surface-skimming (wet-resistant hairs on the wings and ventral tarsi) are shared with the primitive subadult stage of mayflies ("subimagos"; this stage emerges onto the water surface from an aquatic nymph), but not with the more derived adult stage ("imagos"). These traits may be an ancient feature inherited by extant mayflies and stoneflies from a shared surface-skimming ancestor. In combination with ecological factors affecting extant aquatic insects, these results provide a scenario for how and why aquatic insects that swam using thoracic gill plates evolved into the first flying insects. Supported by NSF grant IBN-9317969.

39.12

ONTOGENETIC CHANGE IN THE ENERGETIC COST OF LOCOMOTION IN THE COCKROACH. Elizabeth J. Queathem and Robert J. Full. Department of Integrative Biology, University of California at Berkeley, Berkeley, CA 94720-3140.

The effects of the arthropod molt cycle on pedestrian locomotion have been previously unknown. We measured the energetic cost of locomotion, stride frequency, and ground contact time at 0.31 km/hr in last-instar and adult cockroaches (*Blaberus discoidalis*). The energetic cost of locomotion in adults began high at 6.30 ml O_2/g/km, and declined after the first two weeks of adulthood to about 4.35 ml O_2/g/km. 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 could not be explained by either stride frequency or ground contact time, as neither of these variables changed, so changes in the rate of muscle force development could not have been responsible for increased cost in adults on day one. We also measured maximal leg force production in adults, and found that leg force/body weight on day one was only half its value on day nine. This might cause an increase in cost for two reasons. Animals may have to recruit a larger percentage of less economical muscle fiber types, or reduced skeletal stiffness may cause muscles to do additional work before ground reaction forces can be generated.

39.13

INSTANTANEOUS JOINT POWER OF RUNNING ROACHES. Robert J. Full, Rodger Kram and Ben Wong. Univ. of California, Berkeley 94720

Integration of isolated muscle function with whole-body mechanics in complex systems requires analysis at a common point such as the joint of animals with jointed framework skeletons. To estimate the instantaneous power at each joint of every leg, we determined the product of joint angular velocity and the moment or turning force at the joint produced by the muscles of the death-head cockroach, *Blaberus discoidalis*, running at 20 cm/sec. Joint moment was derived from three dimensional kinematics and ground reaction forces. Muscles at different joints of a given leg did not all operate in the same way, even during the stance phase. Muscles produced or absorbed energy or did a bit of both. Muscles of a given joint could produce energy in one leg and absorb energy in another. Net positive power generation over a stride was 18 W/kg for femoral extensor muscles, a value comparable to our simulation estimates. Estimates of muscle power from joint power were comparable to estimates derived from isolated muscle and can provide a link between leg dynamics and muscle function. ONR Grant NOOO14-92-J-1250.

ONTOGENY OF CARDIOVASCULAR SYSTEMS

40.1

ONTOGENY OF CARDIAC FUNCTION IN CRUSTACEANS. **John I. Spicer & David Morritt Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2UQ, U.K.**

The ontogeny of cardiac function has been studied in three species of crustacean arthropods. In the brine shrimp *Artemia franciscana* heart formation and consequently the ontogeny of cardiac function was postembryonic. Initially heart rate (f_H) increased with increased development, and was insensitive to changes in environmental temperature (24 to 32°C). When differentiation of cardiac tissue neared completion an inverse relationship between f_H 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^{-9} - 10^{-3} M), but there was no effect on f_H. In both the cladoceran *Daphnia magna* and the amphipod *Gammarus duebeni* although cardiac function was present before hatching, the same pattern of f_H changes was observed: an initial steep increase followed by a slow decrease with development. The f_H of early embryonic amphipods was not affected by exposure to waterborne ACh although f_H of pre-hatch individuals was affected. Interestingly f_H 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 f_H may be independent of external influences.

40.2

HEART RATE DURING DEVELOPMENT IN A REPTILE: EFFECT OF TEMPERATURE. G.F. 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 (*f*) during development remains undescribed. This study examined *f* and body and heart mass over the course of development in snapping turtles (*Chelydra serpentina*) at 24 and 29 C. Heart rate early in development (<25 d) was significantly higher in eggs incubated at 29 C. After day 25 *f* in 29 C eggs dropped steadily until hatching. Heart rate in 24 C eggs remained relatively steady during most of incubation and showed a slight decline near hatching. Heart rate just before hatching was similar at both temperatures. The heart mass/body mass ratio (H/B) remained steady for most of incubation and then declined significantly at both temperatures as hatching approached. The decrease in *f* and H/B 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 LOCUS 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 Leghorns (about 295-305 bpm from day 8 to day 20 of incubation), c^a/c^a and other 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 16-20. This chicken strain may be a useful animal model for determining how heart rate anomalies 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 Weidner. 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-blood barrier protected against fluid accumulation caused by increased microvascular filtration (Weidner et al. Resp. Physiol.91:125-136,1993). In this study we employed confocal microscopy to elucidate the normal pathway of lymphatic drainage in the lung of Gallus. White Leghorn cocks (2-4 kg body weight) were anesthetized 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.

40.5

NONINVASIVE DETERMINATION OF INSTANTANEOUS HEART RATE OF DEVELOPING AVIAN EMBRYOS. Hiroshi Ono*, R. Akiyama*, M. A. Haque*, J. Pearson* and Hiroshi Tazawa. Muroran Institute of Technology, Japan.

The heart rate of developing avian embryos within the egg shell is determined by measuring electrocardiogram, impedance-cardiogram, ballistocardiogram (BCG), or acoustocardiogram (ACG). While the former two are electrical signals measured invasively by inserting needle electrodes into the egg through the shell, the embryonic heart rate can be determined even in an early period of incubation. Both BCG and ACG are cardiogenic signals detected from the outside of the shell, offering noninvasive determination of embryonic heart rate during late period of incubation. Although a condenser microphone has to be installed air-tightly on the shell, we found that ACG signal was less contaminated with somatic movements of embryos than BCG. 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.

40.6

TRAJECTORY OF AVIAN EGG BALLISTOCARDIOGRAM. James Pearson*, H. Ono*, M.A. Haque* and Hiroshi Tazawa. Muroran Institute of Technology, Japan.

Impact and recoil of cardiac contractions of avian embryos move the egg minutely. The minute ballistic movements of the egg were detected by various means from the eggshell and referred to as ballistocardiogram (BCG) of the egg (J. Appl. Physiol. 67: 478-483, 1989; Med. & Biol. Eng. & Comput. 27: 580-586, 1989; 29: 393-397, 1991; 31: 129-134, 1993). In the present study, using two laser displacement meters, we measured simultaneously the BCG from two points of the eggshell which faced each other on the short or long axis, or were located at right angles and plotted trajectory of BCG every 5 msec with reference to ECG on a computer monitor to elucidate two-dimensional cardiogenic ballistic movements of the chicken eggs. The eggs were placed either in a horizontal position or in an upright position. The cardiogenic displacement of the egg was 1-2 μm and it tended to be larger in the vertically positioned eggs than in the horizontally positioned eggs. The trajectory pattern of BCG was displayed (and printed, if necessary) every one cardiac cycle. The horizontally positioned eggs mainly swung along the short axis (breadth) of the egg with a few exceptional movements. In contrast, the cardiogenic movements of the vertically positioned eggs were multi-directional and the patterns of trajectory tended to vary with the lapse of time. The trajectory clearly shows that chicken embryos change their posture both slowly and quickly in the egg with time.

40.7

EMBRYONIC DEVELOPMENT OF STORED AVIAN EGGS AND UNTURNED EGGS. Md. Aynal Haque*, H. Ono*, J. Pearson* and Hiroshi Tazawa. Muroran Institute of Technology, Japan.

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 (oxygen consumption per heartbeat) 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 unturned eggs, indicating delay of development of the embryos and autonomic nervous control of the heart. (2) Then, the wet and dry mass of embryos were determined after oxygen consumption measurement of eggs 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.

40.8

CORONARY BLOOD FLOW IN SWIMMING RAINBOW TROUT: EFFECTS OF HYPOXIA, AND CHOLINERGIC AND β-ADRENERGIC ANTAGONISTS A. Kurt Gamperl*, Michael Axelsson and A.P. Farrell. Dept. of Biol. Sci., Simon Fraser Univ. Burnaby, B.C. Canada. V5A 1S6

To further examine the relationship between coronary perfusion and cardiac performance, cardiac output ($\dot{Q}$), coronary blood flow ($\dot{q}_{cor}$), and dorsal aortic blood pressure (P_{da}) were measured in resting and swimming (0.5 - 1.0 bl s^{-1}) rainbow trout (*Oncorhynchus mykiss*) during normoxia and hypoxia ($P0_2$ approx. 10 kPa). In normoxic trout, stepwise changes in cardiovascular variables were observed as the swimming speed was incrementally increased. At 1.0 bl s^{-1} $\dot{q}_{cor}$ and cardiac power output had both increased by approximately 110%, and coronary artery resistance (R_{cor}) had decreased by 35%. During hypoxia, resting $\dot{q}_{cor}$ was 30% higher and R_{cor} was 20% lower, as compared with normoxic values. In hypoxic swimming trout, the maximal levels of $\dot{q}_{cor}$ (155% increase) and R_{cor} (50% decrease) were recorded by 0.75 bl s^{-1}. In contrast, cardiac power output and $\dot{Q}$ 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 $\dot{q}_{cor}$ 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 $\dot{q}_{cor}$ in swimming trout is approximately 150%. In addition, the cholinergic and β-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 R_{cor}. Propranolol injection into atropine treated trout increased resting R_{cor}, and reduced their ability to increase $\dot{q}_{cor}$ and to decrease R_{cor} when swimming at 1.0 bl s^{-1}.

40.9

How hearts, percentage of red muscle, and heat exchangers vary with fish size in juvenile black skipjack tuna (*Euthynnus lineatus*). Kathryn A. Dickson, Jeevan Daniels*, Laura Emge*, Reni Fox*, and Noel Johnson*. California State University, Fullerton, CA 92634

Tunas are unique among teleost fishes in using metabolic heat retained by counter-current heat exchangers (retia) to elevate muscle temperatures significantly above water temperature (endothermy). This study determined how aerobic tissues and the retia vary ontogenetically in the black skipjack tuna *Euthynnus lineatus*. Post-flexion larvae and early juveniles [10-20 mm fork length (FL)] were collected by nightlighting and were raised to larger sizes at the Inter-American Tropical Tuna Commission laboratory at Achotines Bay, Panama. Whole hearts were removed from the pericardial cavity of formaldehyde-fixed fish and were weighed on an analytical balance. Hearts comprised 0.44% of body weight (18.8-244.0 mm FL, 0.06-264.5 g), and heart weight scaled isometrically. Using video analysis (Raster-Ops frame grabber and NIH Image software) of 10-15 um thick transverse sections of paraffin-embedded hearts from fish 63-157 mm FL, we found that the outer compact layer comprised 60%, and the inner spongy layer comprised 40%, of ventricle cross-sectional area. Using a computer-interfaced digitizing tablet and Jandel JAVA software to analyze photomicrographs of fish transverse sections (10-15 um thick), the relative area comprised of red myotomal muscle fibers (%RM) was estimated. At 45-50% of FL, red muscle comprised 16.0% of total muscle cross-sectional area in black skipjack 54.8-129.0 mm FL, and %RM did not increase significantly with body size. Transverse sections of tunas 35.5-234 mm FL were examined to determine the minimum size at which central and lateral retia are present, and how they vary with fish size. All individuals ≥ 108.7 mm FL had central rete blood vessels and those ≥ 129 mm FL had lateral rete blood vessels, and rete size increased with fish size.

40.10

EVOLUTIONARY DEVELOPMENT OF THE CARDIOVASCULAR SUPPLY TO THE VERTEBRATE BRAIN Gregory K. Snyder. Dept of EPO Biology, University of Colorado, Boulder CO 80309-0334, USA.

The development of capillary networks was an integral part of the evolutionary development of a cardiovascular system that provides essential nutrients to metabolizing cells and removes wastes from those cells. In systemic tissues the role of capillaries is passive and exchange is by simple diffusion. In brain the tissues are "vigorously isolated" from blood by capillary endothelial cells or surrounding glia which selectively exclude some materials and actively transport other materials. In this way a unique microenvironment surrounds neural cells. However, lipid soluble substances may readily pass through the lining barrier. During hypercapnia, for example, the brain may be subject to the same acid-base challenges found in systemic tissues.

I used ^{31}P-magnetic resonance spectroscopy and the transmembrane distribution of DMO to investigate the effects of hypercapnia on intracellular pH in brain of two lizard species; *Anolis equestris* and *Dipsosaurus dorsalis*. Plasma PCO_2, 3.3 ±0.1 kPa, and plasma pH, 7.52 ±0.01, in control *D. dorsalis* were not significantly different from the values, 2.8 ±0.2 kPa and 7.59 ±0.02, for *A. equestris*. However, control values of brain pHi, 7.11 ±0.02, for *D. dorsalis* were significantly higher than brain pHi of *A. equestris*, 7.02 ±0.02. In addition, brain pHi of *D. dorsalis* decreased significantly following hypercapnia while in *A. equestris* brain pHi was unchanged. These findings were independent of the methods used to determine pHi. Thus, the whole problem of intracellular pH regulation is more complex than previously thought and that the results are influenced to a large degree by the species studied.

This work is supported by NSF DCB8818647 and NIH HL32894.

40.11

BIOMECHANICS AND BIOPHYSICS OF NECK VEINS IN GIRAFFE AND OSTRICH DURING POSTURAL MANEUVERS.

Ronald W. Millard, Anthony J. McGoron*, Douglas L. Armstrong* and James W. Hicks. U. of Cincinnati, Cincinnati, OH, 45267-0575 and Henry Doorly Zoo, Omaha, NE.

Venous blood volume determined by composition of blood vessel wall and surrounding tissues is influenced by hydrostatic pressure and autonomic nervous system changes. The ostrich jugular vein lies relatively freely under a loose skin while that of the giraffe lies relatively firmly tethered under a thick skin and deep within fascia layers of adjacent neck muscles. Veins in the head and neck are stressed by high gravitational forces in the head down position and unloaded in the head up position. We determined the size and shape of the jugular vein of giraffe (n=1) and ostrich (n=1) at several locations where blood pressure(P) was also measured. Intravascular ultrasound imaging and pressure tip transducer catheters were advanced up to 1m toward the heart from insertion sites 10 to 20 cm from the angle of the jaw. Venous lumen cross-sectional area(A) normalized to A at P=0 mmHg was related to P during head up/head down postures and during proximal venous occlusion. Data indicate that *in vivo* venous stressed area limit is much greater in ostrich (~16) and giraffe (>16) than predicted from studies on *ex vivo* canine jugular vein (~1) or thin walled latex tubing(~1) (Moreno et al., Circulation Res. 1970). In addition, the venous P gradient in the jugular vein of the head down giraffe but not the ostrich appeared to be less than predicted by hydrostatic equations. We conclude that venous vascular or extravascular structures are highly important in A and P adjustments to posture induced gravitational changes. A yet to be identified feature in the giraffe appears to reduce cranial venous P in head down.

40.12

HIGH COOPERATIVITY OF HEMOGLOBIN-OXYGEN BINDING IN PIG EMBRYONIC RED CELLS.

Robert A.B. Holland, Sandra L. Butler*, Susan J. Calvert*, and Robert J. Love*. School of Physiology and Pharmacology, Univ of New South Wales, Sydney 2052; and Univ of Sydney Dept of Animal Health, Camden N.S.W., 2570, Australia.

We have previously shown that the blood oxygen equilibrium curves (OECs) of embryonic-type blood in three marsupial species, and of embryonic blood in a eutherian mammal (rabbit), give a value of the Hill coefficient (nH) significantly greater than 4 above about 50% saturation. This coefficient is an index of cooperativity between hemoglobin subunits and so values of nH greater than 4 indicate that the functional unit of hemoglobin is larger than a tetramer. The pig (*Sus scrofa*) was studied as a second eutherian mammal to see how general was the aggregation of Hb tetramers in embryonic blood. Blood was obtained from 8 pig embryos taken from mothers 29-30 days after mating. Over 98% of the red cells were nucleated and isoelectric focussing showed the presence of embryonic hemoglobins. OECs were determined on blood or red cells by a thin film method (modified Hemoscan) at 38.5°C and at Pco_2 = 42 torr. In all embryonic blood nH was greater than 4 in the upper part of the OEC (mean nH at 42 torr Pco_2 was 4.72, SE = 0.16). The adult nH was in the range 2.8-3.1, similar to what others have previously reported. The P50 of the embryonic blood at 42 torr Pco_2 was 27.8-35.8 compared with 34.8-35.7 torr for adult blood. The results indicate that the embryonic blood has aggregation of Hb tetramers and an O_2 affinity similar to or a little higher than that of adult blood. (Supported by a grant to RABH from the Australian Res. Council).

ADAPTATIONS TO EXTREME ENVIRONMENTS

41.1

LIVING AT 0°C-RESPIRATORY GAS EXCHANGE, ACID-BASE STATUS AND RATES OF PROTEIN SYNTHESIS IN THE ANTARCTIC ISOPOD, *GLYPTONOTUS ANTARCTICUS*

N.M. Whiteley, E.W.Taylor and A.J. El Haj. Biological Sciences, University of Birmingham, Birmingham, U.K.

Glyptonotus antarcticus is a common benthic isopod found around the coast of Antarctica where water temperatures remain at 0±2°C. *Glyptonotus* has a long evolutionary history in this environment, and as a consequence is strictly stenothermal with a maximum temperature limit for survival of 6°C. Mean oxygen uptake rate in resting *Glyptonotus* was 11.19±0.74(16) μmol kg^{-1} min^{-1} and mean whole body rates of fractional protein synthesis was 0.27±0.06(5) % day^{-1}. Comparison of these rates, compensated for size, to the values obtained from a temperate isopod, *Idotea rescata*, acclimated to 4 and 13°C, indicates that *Glyptonotus* does not show metabolic cold adaptation or elevated protein synthesis levels at 0°C. In addition haemocyanin levels in *Glyptonotus* were low and only played a minor role in the transport of oxygen to the tissues as 70% was carried as dissolved oxygen. Mean haemolymph pH was 7.88±0.01(6) which was relatively acidotic when compared to Antarctic fish blood and to the value predicted from the concept of constant relative alkalinity. Supported by NERC, GR3/8329. Animals supplied by the British Antarctic Survey.

41.2

DIURNAL THERMAL AND ELECTROMYOGRAPHICAL RESPONSES TO CHANGING AMBIENT TEMPERATURES IN THREE SPECIES OF HUMMINGBIRDS WITH DIFFERENT BODY MASSES.

Günther Warncke. Institute for Neurophysiology of the University of Cologne, Robert-Koch-Str.39, 50931 Cologne, Germany.

In three neotropical hummingbird species (Amazilia tzacatl (n=5), average body mass: 3,9 g, Colibri coruscans (n=5): 7,9 g, Patagona gigas (n=3): 19,9 g) core temperature (Tco), electromyographical activities (EMG) and respiratory frequency (rfr) were simultaneously and continuously recorded under laboratory conditions (LD 12:12, ambient temperatures (Ta): daytime, 22-24°C; nighttime, 6-8°C; nectar ad lib.) over 7 to 14 consecutive days. Each of the trochilids revealed a body mass related regulatory pattern in all three parameters studied. During the day Amazilia tzacatl exhibited average electromyographical activities of 590 μV (max. amplitude). In the larger Colibri coruscans, these values reached 470 μV during light regimes, Patagona gigas, however, only reached 160 μV. Amazilia tzacatl did not enter torpor at night but a numbness-like state, indicated by a relatively high Tco of about 36,2°C and by continuous muscle activity reaching up to 280 μV. Whereas the heavier Colibri coruscans underwent torpor at night. Prior to this energy saving state, core temperature dropped in Colibri coruscans from 42,1°C to 36,3°C (euthermic, shallow sleep), and decreased steadily to 8,2°C (time span required to drop from maximum to minimum Tco: 2.5 h). This lowest temperature value was kept constant by means of a sporadically appearing but then low and continuous EMG of 40 μV. During the nocturnal stages from shallow sleep to torpor Colibri coruscans lowered the rfr from 120$xmin^{-1}$ to 38$xmin^{-1}$. Rfr values in deep torpor could not be recorded.

The largest hummingbird, Patagona gigas, also exhibit torpor at night. Related to the high body mass and its great capacity of energy saving, Tco dropped in this species from 42,1°C to 39,6°C and then to 34,9°C (wakefulness, euthermic to hypothermic sleep), and thereafter decreased steadily to 14,2°C or 9,1°C in torpor. In 78 percent of all torpid stages Patagona only entered torpor at Tco=14,2°C. In contrast to the smaller species the lowest temperature value in Patagona was not maintained by means of a sporadically appearing low and continuous EMG, but by two different patterns of EMG. Brief bursts appeared when entering torpor stage of Tco=14,2°C. Entering deep torpor the birds exhibited a low intermittent EMG (30-50 μV) when Tco remained under 9,1°C. During the nocturnal stages from sleep to torpor Patagona gigas lowered the rfr to 38$xmin^{-1}$. In deep torpor (Tco=9,1°C) only a rfr of 5$xmin^{-1}$ could be registered.

Arousal from torpor to shallow sleep was accompanied by strong shivering (4200-2300 μV), time span (40-90 min) in all birds. Finally, awaking from sleep was induced by light, after which daytime EMG-activities (160-470 μV) were resumed. Further experiments on Electroencephalogram (EEG) and blood chemistry will be continued in all hummingbirds.

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RESPIRATION AND ACID-BASE

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 92717

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 CO_2. 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 respirometry system, we have measured CO_2 release from individual flies into a stream of dry, CO_2-free air. We found clear examples of DV, the first such observation in a small Dipteran. This behavior is even more interesting, since to date we have observed it only in an outbred population that has been selected over multiple generations for resistance to desiccation. The examination of DV in selected lines of *Drosophila melanogaster* may provide insight into the role of DV in desiccation resistance. Supported by grant US-PHS AG09970.

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 (CO_2) transport in the blood of lampreys is largely dependent on CO_2 carriage within the red blood cell. This in vitro study used both molecular and physiological approaches to determine the factors contributing to this unusual strategy for CO_2 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 cell. However, the distribution of bicarbonate across the red blood cell membrane only became similar to that in the rainbow trout when an ionophore for anions, tributyltin chloride, was added to the suspension. Moreover, the anion exchange inhibitor, 4,4' diisothiocyanatostilbene-2,2' disulfonic acid (DIDS) did not significantly affect bicarbonate distribution in lamprey blood. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of lamprey red blood cell membranes confirmed that the anion exchange protein, Band 3, was extremely limited or absent in sea lamprey red blood cells. Thus, both sodium/proton exchange and the absence of rapid anion exchange contribute to the unique CO_2 transport properties in lampreys.

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42.3

BLOOD RESPIRATORY PROPERTIES OF SOME AIR-BREATHING GOBIES. Nancy M. Aguilar and Jeffrey B. Graham. Scripps Institution of Oceanography, La Jolla, CA, 92093-0204.

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, interfamiliar comparisons do not support this generalization, differences do occur among 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 dissociation curves (ODCs) were generated, using biotonometry, for two gobies, Periophthalmus barbarus, an amphibious fish and Gillichthys mirabilis, a facultative air-breather. ODCs were compared between 20 and 35°C, at both pH 7.6 and 7.8. Oxygen capacity and blood pH were determined at 25°C. The ODC of P. barbarus is right shifted (P_{50}=35 at 30°C) relative to G. mirabilis (P_{50}=18.5 at 30°C). P. barbarus has a higher hematocrit and higher hemoglobin concentration than G. mirabilis. Expansion of the approach to include other goby species with various air-breathing specializations will furthur define the effect of the evolution of air-breathing on blood characteristics on this family.

42.4

RESPONSE OF REPTILIAN INTRAPULMONARY CO_2 RECEPTORS TO NH_4Cl BEFORE AND AFTER ACETAZOLAMIDE ADMINISTRATION. Richard D. Tallman, Jr. School of Allied Medical Professions and Department. of Physiology, The Ohio State Univ., Columbus, Ohio 43210

The Pine snake (*Pituophis 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 airway PCO_2. Earlier studies have shown that IPC in birds and lizards respond to an infusion of acetazolamide by a gradual and sustained increase in discharge. In addition, NH_4Cl has been shown in a variety of preparations to induce an increase in intracellular pH during the infusion period and a drop in pH below control upon cessation. The purpose of the present study was to determine the response of IPC to NH_4Cl infusion before and after the blockade of carbonic anhydrase with acetazolamide.. Pine snakes (avg. wt. 390 gm) were anesthetized (pentobarbital 24 mg/kg S.Q.) and unidirectionally ventilated. For drug infusions, a non-occlusive catheter was placed in the pulmonary artery close to the heart. In most, but not all IPC, a bolus injection of 10 mg NH_4Cl resulted in a rapid increase in discharge followed by a decrease or silence lasting up to 60 sec. Other IPC responded to NH_4Cl with a slow decrease in discharge. Acetazolamide (50 mg/kg) caused a slow but sustained increase in discharge of all IPC. The responses to NH_4Cl were virtually unaltered by the acetazolamide although the CO_2 sensitivity was greatly diminished. The results of the present study support the theory that IPC operate through an intracellular pH sensitive mechanism.

42.5

OXYGEN CONSUMPTION, NITROGEN EXCRETION, BRAIN MONOAMINES AND BRAIN ENERGY METABOLITES IN COPPER EXPOSED CARP. Gudrun De Boeck[1], Hans De Smet[1]*, Göran E. Nilsson[2] and Ronny Blust[1]. [1]University of Antwerp (RUCA), B-2020 Antwerp, Belgium; [2]University of Uppsala, S-752 36 Uppsala, Sweden.

Juvenile common carp (15-30g) were exposed to copper levels of 0.22, 0.34 and 0.84 μM during one week. O_2 consumption and nitrogen excretion were determined repeatedly and critical O_2 concentrations for O_2 consumption as well as for ammonia excretion were determined after one week of exposure to copper. Also serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxyindoleacetic acid (5-HIAA, the main 5-HT metabolite), dopamine (DA), ATP, ADP and AMP were measured in telencephalon, hypothalamus and brain stem after one week of exposure. In addition, lactate concentrations were determined in brain stem. O_2 consumption dropped significantly immediately after exposure to 0.34 and 0.84 μM of copper whereas nitrogen excretion remained stable. After one week of exposure to 0.34 μM of copper the O_2 consumption showed an apparent recovery, while the ratio of O_2 consumption rate and ammonia excretion rate (O:N ratio) did not. At a copper concentration of 0.84 μM, no recovery was observed. The critical O_2 concentration for O_2 consumption shifted from 1.4 mg l^{-1} in copper free water to 3.9 mg l^{-1} at a copper concentration of 0.34 μM. At 0.84 μM, regulation of O_2 consumption was lost. Also ammonia excretion showed a critical O_2 concentration. For this nitrogen excretion the loss of regulation already occurred at copper concentrations of 0.34 μM. For the O:N ratio, no critical O_2 concentration was found. In telencephalon, dose dependent falls in 5-HT and DA levels were observed, with close to 50% losses of these neurotransmitters at the highest copper concentration. For brain stem, a significant decrease in 5-HT and DA could be seen in the group exposed to 0.84 μM, while in hypothalamus only 5-HT levels were significantly lower at this highest copper concentration. No changes in either 5-HIAA, AMP, ADP, ATP or adenylate energy charge could be observed in any of the brain parts. Also lactate levels in brain stem were stable. 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 least temporary impaired at the two higher copper concentrations, while nitrogen excretion remains stable. It is also shown that a critical O_2 concentration for ammonia excretion exists in carp which, like the critical O_2 concentration for O_2 consumption is affected by exposure to copper.

42.6

EFFECTS OF LONG TERM HYPOXIA ON BEHAVIOURAL THERMOREGULATION IN ADULT RATS. P.B. Frappell and F.L. St Clair. School of Zoology, La Trobe Univ., Melbourne, Victoria, Australia, 3083.

In small adult mammals, it is well established that acute hypoxia decreases both metabolic rate ($\dot{V}O_2$) and body temperature (Tb). Furthermore, hypoxic animals have been shown to behaviourally thermoregulate at a lower Tb, suggesting that their thermoregulatory set-point is shifted. To determine if the change in the set-point is temporary, behavioural thermoregulation by 6 Spraque-Dawley rats, implanted with temperature transmitters for measurement of Tb, were studied under normoxia (21% O_2) and hypoxia (10% O_2) for 4 days (food and water *ad libitum*) in a modified temperature gradient that permitted the determination of $\dot{V}O_2$. Normoxic rats selected a mean ambient temperature (Ta) of 24.2 ± 3.2 (SD)°C while maintaining Tb at 37.6 ± 0.6°C and $\dot{V}O_2$ at 26.4 ± 3.3 ml/min/kg. At the onset of hypoxia (0-6 hr), rats selected a significantly warmer Ta (28.1 ± 3.7°C) but by 8 hr this had reduced to 26.4 ± 2.5°C; still above that observed in normoxia and where they remained. Hypoxia decreased Tb (36.6 ± 0.7°C) and $\dot{V}O_2$ (21.4 ± 1.9 ml/min/kg) but after 32 hr in hypoxia, both Tb and $\dot{V}O_2$ had returned to pre-hypoxic levels. These findings support the notion that a reduction in $\dot{V}O_2$ is an immediate emergency-type response to hypoxia and one that is not necessarily desirable in chronic conditions. The maintenance of an elevated Ta, after $\dot{V}O_2$ and Tb had returned to pre-hypoxic values, suggests that the selection of a higher Ta is an attempt to reduce the increased thermolysis that occurs with hypoxia by diminishing the Tb-Ta gradient rather than a resetting of the thermoregulatory set-point.

42.7

THE SPONTANEOUS COUPLING OF METABOLISM AND VENTILATION IN ONE-WEEK OLD RATS. Chikako Saiki and Jacopo P. Mortola, Dept.Physiology, McGill Univ., Montreal, Canada.

One-week old rat pups, mother-reared under 12:12 hr light-dark cycle (light on from 7:00-19:00) presented high metabolic rate at night, and low in the morning. We questioned to what extent these variations were met by changes in ventilation ($\dot{V}_E$). Oxygen consumption ($\dot{V}O_2$, measured by a flow through method) and $\dot{V}_E$ (by airflow plethysmography) were measured on 6-day old pups at 7:30, 19:30, and again at 7:30 of the next day. In normoxia, all values were higher at 19:30 than at 7:30, the difference being more pronounced at 29°C than at thermoneutrality (33°C). At all hours, $\dot{V}_E$-$\dot{V}O_2$ ratio remained constant (36.5 at 33°C, 32 at 29°C), because changes in $\dot{V}_E$ perfectly matched those of $\dot{V}O_2$. In acute hypoxia (inspired O_2=10%), $\dot{V}O_2$ and $\dot{V}_E$ dropped significantly from normoxia, and more so at 19:30 than at 7:30, resulting in no difference in $\dot{V}O_2$, $\dot{V}_E$ or $\dot{V}_E/\dot{V}O_2$ among hours. These results indicate that in the 1-week old rat pup $\dot{V}_E$ control is sufficiently developed to finely track changes in metabolic rate, whether spontaneously occurring during the day or induced by hypoxia. (Quebec Lung Association)

42.8

EFFECTS OF HIGH ALTITUDE PULMONARY DEVELOPMENT ON VO_2max IN THOROUGHBRED HORSES: EVIDENCE FOR SYMMORPHOSIS. J. H. Jones, B. L. Smith*, N. L. Couper* and M. Kann*. Dept. of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616.

Thoroughbred horses achieve relatively high mass-specific rates of oxygen consumption (VO_2max/kg) with evidence of limitations appearing in all components of the O_2 transport system: peripheral tissue diffusion, circulatory convection, pulmonary diffusion, and ventilatory convection. These findings suggest that in these highly selected aerobic athletes malleable elements of the respiratory system (mitochondria and capillaries, ventilatory and cardiac muscles) have hypertrophied to match the conductance of the least malleable element in the system, pulmonary diffusion capacity for oxygen (DL_{O_2}). We hypothesized that increasing relative DL_{O_2} by raising foals in chronic cold and hypoxia at high altitude (HA) would increase VO_2max/kg. We raised two foals at 2,600 m for two years and compared their VO_2max/kg at sea level (SL) as three year olds with those of three age-matched foals raised at SL. VO_2max/kg in HA horses (3.10 ± 0.04 (SE) and 2.88 ± 0.06 (SE) ml O_2 (STPD) s^{-1} kg^{-1}) were higher than for SL (2.61 ± 0.02 (SE)) and exceeded the 95% confidence limit. Although mass-specific VO_2max was higher in HA horses, whole-body VO_2max were similar (1359.5 ± 3.9 (SE) and 1363.5 ± 5.8 (SE) ml O_2 (STPD) s^{-1} kg^{-1}) to those of SL horses (1396.5 ± 11.8 (SE)), suggesting that HA and SL horses had similar absolute DL_{O_2}, but with smaller bodies in HA (439 and 474 kg) than in SL (528 ± 3.5 (SE) kg). These results support the hypothesis that in a highly selected aerobic athlete, malleable respiratory structures hypertrophy to match the conductance of the least malleable structure, the lung. Supported by UCD Equine Research Laboratory with contributions from the Oak Tree Racing Association, California Satellite Wagering Fund, and private donations.

42.9

POSTURAL-VENTILATORY INTEGRATION IN THE PANTING DOG.

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

Cineradiography and pneumotachography 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 respiratory air flow. Ventilatory 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 represent a potentially significant, but previously unrecognized addition to the work of breathing. Above respiratory frequencies of 1.5 Hz., respiratory airflow is generally in phase with diaphragmatic 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 lung 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 phase of these lobes from synchronous to fully asynchronous probably involves modulation of the impedance of the anterior chest walls overlying the apical lobes through changes in the recruitment patterns of the muscular suspensory slings of the thorax.

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42.10

GAS COMPOSITION IN MIDDLE EAR CAVITY vs SUPERIOR VENA CAVA BLOOD OF THE GUINEA PIG.

A. Ar[1], M. Luntz*, H. Mover*, D. Levi*, M. Harell* and J. Sadé*. Departments of Zoology[1] and Biomed. Engineering, Tel Aviv Univ., Tel Aviv 69978, Israel.

Middle ear cavity (ME) was modeled as a poorly ventilated gas pocket with rigid walls and close to atmospheric Pb. This predicts almost identical steady-state values for O_2 and CO_2 partial pressures in ME ($PmeO_2$; $PmeCO_2$) and in the venous blood leaving it (PvO_2; $PvCO_2$) respectively, and a pronounced PN_2(+PAr) difference, with high PN_2 in ME. However, whether ME gas exchange is limited by diffusion or perfusion, is not established. A calibrated membrane-covered mass-spectrometer probe (suction=2ml/d) was inserted via the bulla into the ME of 18 anesthetized guinea pigs, and the opening sealed. In 4 animals a 2nd probe was introduced into the superior vena cava. Gas composition was monitored until steady-state was achieved. Pressures were calculated using dry gas fractions, Pb and vapor saturation. The PvN_2(+Ar) was assumed to equal that of alveolar gas and readings of other gases in blood to be proportional to it. Values (Torr) were: $PmeO_2$=48±8SD; $PmeCO_2$=66±11SD; $PmeN_2$=599±10SD; PvO_2=38±2SD; $PvCO_2$=61±2SD; PvN_2=563±4SD. Time constants (TC, sec^{-1}) for steady-state establishment were 13±11SD and 27±17SD for O_2 and CO_2 respectively. The PO_2 difference may reflect mixed sources of blood but the large PN_2 difference is close to predicted. The O_2/CO_2 TC difference is insignificant, not proving large diffusive gas/blood resistances.

42.11

EFFECTS OF CALCIUM DEFICIENT DIET AND ACETAZOLAMIDE ON PORE AND MAMMILLARY KNOB FORMATION IN AVIAN EGGSHELLS.

D. E. Bebout, 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* (Bebout and Hempleman. *Respir. Physiol.* 95: 11-20, 1994) calcium deficient diet increased eggshell water vapor conductance (G_{H2O}) 30% and was accompanied by a 21% decrease in eggshell thickness (L). On the other hand, acetazolamide increased G_{H2O} 200% (first day after administration) and was accompanied by an 89% increase in total functional pore area (A_p), though a 36% decrease in L was also evident. The purpose of this study was to investigate the effects of calcium deficient diet and acetazolamide on pore and mammillary knob formation in the same eggshells. Standard point-counting procedures were used to estimate pore number and pore radius. Scanning electron microscopy was used to study mammillary knob ultrastructure. Calcium deficient diet (which had no effects on A_p) had no effects on pore number, pore radius or mammillary knob ultrastructure. However, acetazolamide (which increased A_p 89%) increased pore number 68% from 114±7 to 192±27 pores/cm^2 ($P < 0.01$), had no significant effects on pore radius (12.0±0.4 to 11.7±1.0 μm) and severely inhibited mammillary knob formation. We conclude that calcium deficient diet increases G_{H2O} by eggshell thinning with little effects on pore and mammillary knob formation. On the other hand, acetazolamide primarily increases G_{H2O} by increasing pore number with severe effects on mammillary knob formation. (Supported by NIH HL17731).

42.12

CHANGES IN NET CHARGE ON PLASMA PROTEINS.

M.L. Halperin, S. Vasudevan* and K.S. Kamel. Renal Division, St. Michael's Hospital, Toronto, Canada.

Analysis of a clinical case with metabolic acidosis and a very large anion gap in plasma (AG) led to a major conceptual problem—there appeared to be an accumulation of many anions that defied known metabolites (i.e., not ketoacids, D or L-lactate). Further, their metabolic fate was atypical.

Case: A 62 year old woman presented with a 2 day history of polyuria and polydipsia; to quench her thirst, she drank a large quantity of sweetened beverages. She had a diagnosis of NIDDM, but an oral glucose tolerance test two weeks prior was normal. She was drowsy and had a severe degree of ECF volume contraction. Her blood glucose was 112 mmol/l (2016 mg/dl); plasma HCO_3 and AG were 17 mmol/l and 41 meq/l respectively. Her blood lactate was only 3 mmol/l and ß-hydroxybutyrate was < 1 mmol/l. The patient received insulin and a net of 4 liters of isotonic saline. To examine the fate of the accumulated anions, urine output was collected every 2 hr for 24 hr. Over the 24 hr, the AG declined by 22 meq/l. Anions were not lost in the urine as could be deduced from calculation of the rate of excretion of cations ($Na^+ + K^+ + NH_4^+ + Ca^{++} + Mg^{++}$) vs usual anions ($Cl^- + SO_4^= + PO_4^=$ + ß-hydroxybutyrate$^-$ + lactate$^-$) or in the GI tract as there was no diarrhea or ileus. If the anion was metabolized to HCO_3^-, and taking into account the change in ECF volume, the concentration of HCO_3^- should have risen by 16 mmol/l vs the observed 5 mmol/l. These changes in the AG could not be accounted for by changes in albumin concentration or pH in plasma. Based on HCO_3 and anion balances, the change in the AG seemed to reflect an anion with a volume of distribution that is intravascular. Taken together with our previous studies that demonstrated a rise in the AG induced 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 defend intravascular volume via the Donnan effect during marked ECFV contraction.

42.13

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

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

Acid-base transfers across the teleost gill (Na^+/NH_4^+, Na^+/H^+, and/or Cl^-/HCO_3^- exchange) may be influenced by the concentration of external counter ions. Sculpin (*Myoxocephalus octodecimspinosus*) can compensate for an infused acid load over 12-24 h when in seawater (SW) or dilute (20%) SW, but excretion is impaired in very dilute water (4%). Pre-adaptation to 20% SW allows the animals to recover from the acidosis more quickly. We studied the effect of acid infusion (2 meq kg^{-1} HCl) in pre-adapted fish exposed to low external [Na^+] or [Cl^-] (<2% SW). Sculpin in low Na^+ water, took up H^+ at a rate of 0.27 ± 0.04 mmol kg^{-1} hr^{-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 excreted H^+ at a rate of 0.32 ± 0.06 post-infusion (similar to SW 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 acidosis. Acid excretion was directly related to external [Na^+]. We hypothesize that a transbranchial Na^+/H^+ exchange is operating in opposition to a gill Cl^-/HCO_3^- transfer and that a loss of HCO_3^- continues even in the face of an internal acidosis as long as significant external Cl^- is available. Funded by NSF DCM 86-02905 and Hearst Foundation Support.

42.14

LACTATE TRANSPORT ACROSS WHITE MUSCLE CELL MEMBRANES OF RAINBOW TROUT *IN VITRO*.

Yuxiang Wang, Christina, F. Misiaszek*, George, J.F. Heigenhauser, 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 post-exercised white muscle. The transmembrane pH gradient was manipulated by varying perfusate pH (approx. 8.4, 7.9, and 7.4) *via* adjusting HCO_3^- while maintaining Pco_2, and the electrical gradient (E_m) was changed by increasing perfusate K^+ from 3mM to 15mM. Transmembrane lactate distribution is neither pH nor E_m dependent. This suggests that the membrane is very impermeable to Lac and carrier-mediated Lac transport could be involved. Based on this finding, specific blockers: α-cyano-4-hydroxycinnamic acid (CIN), 4-Acetamido-4'-isothiocyanatostilbene 2,2'-disufonic (SITS) and amiloride were used to identify the potential role of various ion transporters in lactate transport. CIN, a blocker of both Lac^-/H^+ co-transporter and Lac^-/HCO_3^-, Cl^- exchange, significantly reduced Lac efflux from post-exercised muscle while SITS, a more specific blocker for Lac^-/HCO_3^-, Cl^- exchange, did not show any significant effect on Lac^- efflux. This suggests that Lac^-/H^+ co-transport 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

ENERGETIC CONSEQUENCES OF INTRACELLULAR ACIDOSIS IN GASTROPOD RADULA PROTRACTOR MUSCLE. C.A. Combs and W.R. Ellington, Dept. of Biological Science, Florida St. Univ., Tallahassee, FL 32306-3050.

We have evaluated the impact of experimental reductions of intracellular pH (pHi) in *in vitro* preparations of the radula protractor muscle (rpm) of the marine gastropod, *Busycon canaliculatum*, using phosphorus NMR spectroscopic approaches. Muscle bundles were superfused in a homebuilt probe and fully-relaxed NMR spectra were acquired at 109.35 MHz. It was possible to "clamp" pHi in various acidotic states by superfusing the muscle with 5, 10 and 15 mM sodium 5,5-dimethyl-oxazolidine-2,4-dione (DMO) in buffered artificial seawater (BASW) (pHe=6.5). Superfusion with DMO resulted in consistent reductions of pHi (7.3 --> 7.0, 6.8 & 6.6, respectively) which persisted for at least 4h. During the acidotic transitions, [arginine phosphate] (AP) decreased and [inorganic phosphate] (Pi) increased in a reciprocal manner and remained constant after the pHi stabilized. The extent of changes in [AP] and [Pi] was directly proportional to the magnitude of the imposed acidosis. $[ATP]_{total}$ remained unchanged in all treatments, while the $[MgATP]/[ATP]_{total}$ ratio declined in direct relation to the extent of the acidosis. Intracellular $[Mg^{2+}]_{free}$ fell incrementally with reduced pHi. All of the above effects were rapidly reversed when the DMO was washed out by changing the superfusate to BASW (pHe=7.8). Intracellular acidosis resulted in net hydrolysis of AP (AP + βH^+--> Arg + Pi; β is a function of pH). This reaction is the net reaction of arginine kinase (AK) and ATPase/synthase. Thus, acidosis produces the expected shift in the AK equilibrium but also produces a disequilibrium of the ATPase/synthase reaction. Supported by NSF grant (IBN-9104548).

42.16

INTERSPECIFIC COMPARISONS OF CAPACITY FOR REGULATION OF pH_i IN MOLLUSCAN MUSCLE. S. Kinsey and W. R. Ellington, Dept. of Biological Science, Florida State University, Tallahassee, FL 32306-3050

The major defense strategies against metabolic H^+ production include intracellular buffering mechanisms as well as ion exchange of acid-base equivalents between intra- and extracellular compartments. It is not altogether clear whether more anoxia-tolerant species have a higher capacity for such regulatory processes. To explore this issue, we have evaluated capacity for regulation of pH_i in cardiac muscle from 4 species of closely related marine gastropod molluscs - *Melongena corona* (high intertidal), *Busycon contrarium* (intertidal/subtidal), *Busycon spiratum* (intertidal/subtidal) and *Fasciolaria tulipa* (primarily subtidal). pH_i was measured via phosphorus NMR spectroscopy at 109 MHz in a homebuilt probe consisting of a 1.9 mm ID muscle chamber with a 5 turn solenoidal coil. When pH of the superfusate (pH_e) was systematically altered, there were minimal changes in pH_i vs. pH_e. Intrinsic intracellular buffering capacity (ß, expressed as μmoles $H^+ \cdot pH^{-1} \cdot ml$ intracellular $water^{-1}$) was determined using pulses of DMO (5,5-dimethyloxazolidine-2,4-dione). In addition, by observing the recovery of pH_i, we estimated the rate of ion exchange [dH^+(or OH^-)/dt = ß x (dpH/dt)]. Cardiac muscle from one of the most anoxia tolerant species, *M. corona*, had the highest ß and dH^+/dt values, consistent with the behavior and micro-habitat of this species. In contrast, only small differences were observed in these parameters in comparisons of the other 3 species. Although the results show some adaptive differences in capacity for regulation of pH_i, it is likely that other facets of the suite of adaptive responses to anoxia are the major determinants of anoxia tolerance (supported by NSF grant IBN-9104548).

42.17

INTRACELLULAR MUSCLE pH RECOVERS RAPIDLY IN GHOST CRABS FOLLOWING EXERCISE TO EXHAUSTION. R.B. Weinstein*[1], J.F. Harrison[2] and R.J. Full[1]. [1]U.C. Berkeley, Berkeley, CA 94720, and [2]Arizona 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 pCO_2 increased from 12.6 to 25.4 torr. Intracellular muscle pH decreased from 7.30 at rest to 6.93 at fatigue. Both hemolymph and intracellular muscle pH returned to resting levels within 30 min of recovery. Hemolymph pCO_2 returned to resting levels within 5 min of recovery. Muscle lactate remained elevated (2.4-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 exercise 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 MALE COLLARED LEMMINGS. Tim R. Nagy, Barbara A. Gower, and Milton H. Stetson. University of Delaware, Newark, DE. 19716

This study was designed to examine the effect of gonadal size on the acquisition of the "winter" phenotype in adult male collared lemmings (*Dicrostonyx groenlandicus*). Lemmings were born and raised to weaning on a preweaning photoperiod (Pre) of either 22L:2D (LD) or 8L:16D (SD). At 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 stage were rated biweekly. The experiment was terminated on week 20 and data were collected. Preweaning photoperiod did not affect ($P = 0.30$) the short photoperiod-induced growth of adult lemmings. Pelage color was significantly ($P < 0.05$) affected by preweaning photoperiod; lemmings from the Pre SD group were whiter at weeks 18 and 20. Bifid claw width was not significantly influenced by preweaning photoperiod. At week 10, lemmings from the Pre SD regimen 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 regimen 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 although testicular regression is conducive to the acquisition of the "winter" pelage, it is not necessary for the acquisition of the complete "winter" phenotype. Thus, collared lemmings appear to have "uncoupled" the seasonal regulation of somatic changes and reproductive function. (Supported by NSF DCB87-14638)

43.2

ANNUAL CYCLE OF PLASMA LIPIDS IN CAPTIVE STRIPED BASS, *MORONE SAXATILIS* . Eric D. Lund*[1], Allen R. Place[1] and Craig V. Sullivan[2]. [1]Center of Marine Biotechnology, Baltimore, MD. 21202. [2]North Carolina State University, Raleigh, NC. 27695-7601.

Protein, lipid and fatty acid concentrations in plasma of eight male and eight female six 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 ± 0.67 SE mg/ml, Range 20.6-86.4) 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 ($p < 0.0001$) lower than those of the males except during early ovarian secondary growth. 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 content of the 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 oocytes are rich in wax esters no wax esters or fatty alcohols 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 (DHA) and 20:5 (EPA) to the gonads.

43.3

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

European starlings (Sturnus vulgaris) are omnivorous passerine birds that include significant amounts of starchy grains (e.g. oats and barley) in their diet when insects and fruit are not available. We predicted that starlings would have high levels of intestinal maltase activity (the main enzyme involved in the last step of the digestion of complex carbohydrates) and the ability to up-regulate intestinal hydrolases (maltase, isomaltase, and aminopeptidase-N) in response to changes in nutrient intake. Birds were fed on three diets: a diet containing 52.5% corn starch, a carbohydrate-free diet, and an insect diet. Diet had a significant effect on intestinal morphology: Birds fed on the carbohydrate-free diet had significantly longer intestines and larger intestinal areas than those fed on either of the two other diets. Diet had a significant effect on aminopeptidase-N and isomaltase activity. Both aminopeptidase-N and isomaltase increased with increased protein and carbohydrate intake, respectively, but the magnitude of the increase was relatively small. Diet had no effect on maltase activity per unit intestinal area, however. Surprisingly, total maltase activity was highest in birds fed on the carbohydrate free diet. This result can be explained by the increased intestinal area exhibited by birds fed on this diet. We used standardized phylogenetic contrasts to compare maltase activity in starlings with that of closely related insectivorous/frugivorous species. Starlings had maltase activities that were not significantly different from those of close relatives that do not eat grain. Maltase activities were 2-4 times higher in five species of granivorous birds than in starlings. We concluded that the dietary flexibility of starlings seems to occur in spite of relatively low intestinal maltase levels and a surprising lack of digestive lability.

43.4

OMNIVORY AND DIETARY PLASTICITY ARE NOT NECESSARILY CORRELATED: DIETARY MODULATION OF INTESTINAL ENZYMES IN FOUR BIRD SPECIES. [1]Enrique Caviedes-Vidal, [1]Daniel Afik, [2]Carlos Martínez del Río and [1]William H. Karasov. [1]Dept. of Wildlife Ecology, University of Wisconsin-Madison, WI 53706 ;[2]Dept. of Ecology and Evolutionary Biology, Princeton University, Princeton, N.J. 08544-1003.

Many bird species exhibit temporal switches in the diet and thus in the nutrients predominating in their food intake. Arguments of economical design have been advanced suggesting that in the diet-switching animals, hydrolase expression should be modulated in relation to this temporally varying intake levels of different nutrients. We tested this hypothesis in four omnivorous bird species (*Gallus gallus, Sturnus vulgaris, Passer domesticus,* and *Dendroica coronata*) fed on diets with contrasting carbohydrate and protein composition. We measured the expression of three membrane-bound disaccharidases (maltase, sucrase, and isomaltase) and one protease (aminopeptidase-N). All four species demonstrated a significant increase in aminopeptidase-N when fed on high-protein diets. The ability to modulate disaccharidases when fed on high carbohydrate diets, in contrast, varied among species: *G. gallus* and *P. domesticus* exhibited a physiologically significant increase in disaccharidase activities, whereas *S. vulgaris* and *D. coronata* did not. These results cast doubts on the generality of the notion that omnivory and plasticity of digestive function are correlated. In the three species studied which exhibit sucrase activity (*G. gallus, P. domesticus,* and *D. coronata*) sucrase and maltase activity were tightly and linearly correlated. This correlation suggests the hypothesis that in avian species, a significant fraction of maltase activity is the result of non-specific activity of sucrase. Purification and characterization of avian sucrase support the above hypothesis.

43.5

PASSIVE ABSORPTION OF GLUCOSE IN THREE BIRD SPECIES: MEDIATED GLUCOSE UPTAKE ALONE CANNOT ACCOUNT FOR TOTAL GLUCOSE ABSORPTION. Daniel Afik, Enrique Caviedes-Vidal and William H. Karasov. Department of Wildlife Ecology, University of Wisconsin-Madison, WI 53706.

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 have been selection against reliance on passive absorption in favor of the specificity of absorption resulting from specific transport proteins in the intestinal brush border. We tested the hypotheses 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 nectarivore - *Trichoglossus haematodus*, a granivore - *Passer domesticus*, and an insectivore/frugivore - *Dendroica coronata*) fed on diets with varying carbohydrate compositions. We measured 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 adopted from pharmacokinetics. None of the species increased mediated glucose uptake on a higher carbohydrate diet Estimates of mediated D-glucose uptake summed over the small intestine length were less then 10% of the whole animal absorption rate *in vivo* in all three species. Passive absorption of L-glucose (the stereoisomer that does not interact with the Na^+/glucose cotransporter) measured *in vivo* could explain 30-80% of the whole animal absorption rate in the three species, confirming that nonmediated absorption can be substantial. The passive pathway appears to provide birds with a digestive system that responds quickly to varying sugar load and that is energetically-inexpensive to maintain, but might increase vulnerability to toxins. Supported by NSF BSR9020280 and IBN9318675.

43.6

SEPARATION OF ACTIVE AND PASSIVE UPTAKE OF METALS AT FISH GILLS THROUGH MANIPULATION OF FISH METABOLIC RATE
Richard Playle, Nancy Janes*, and Rob Macdonald*.
Wilfrid Laurier University, Waterloo, Ontario, Canada. N2L 3C5.

Active metal uptake processes at fish gills are temperature dependent, because fish metabolic rate changes as temperature is increased or decreased (Q_{10} = 2-3). Passive uptake (e.g. diffusion through the gills) is essentially temperature independent over temperatures tolerated by trout, because diffusive flux is dependent on *absolute* temperature. That is, for an increase in temperature from 10° to 20°C (with about a doubling of metabolic rate) there is, in theory, only a small (4%) increase in diffusive flux. A complicating factor is increased ventilation (V_w) to match O_2 demand as metabolic rate increases, but V_w can be held constant by increasing the O_2 content of the water. Silver (Ag) is an ideal metal to use in metal uptake experiments, because of its very low background concentration in gills and blood of rainbow trout (Oncorhynchus mykiss). Measurement of Ag accumulation on gills of small (1-3 g) trout, and Ag passage through gills into blood of larger (~200 g) trout, via dorsal aorta cannulation, are methods currently used by us to separate active and passive metal uptake at fish gills. Gill and blood Ag concentrations are measured by graphite furnace atomic absorption spectroscopy.

43.7

TEMPERATURE AND THYROID HORMONE LEVELS DURING INCUBATION INFLUENCE METABOLIC RATE AND THERMAL CHOICE OF JUVENILE SNAPPING TURTLES. S. O'Steen. Dept. Ecology and Evolution, Univ. of Chicago, IL. 60637

Temperature acclimation can influence energy use rates and thermal preference of adult reptiles. I hypothesized that egg incubation temperature would influence energy use and thermoregulation of juvenile reptiles, and that this influence might be mediated by thyroid hormones. Eggs of the snapping turtle *Chelydra serpentina* were incubated at 21.5, 24.5, 27.5 or 30.5°C. Metabolic rate, measured as oxygen consumption at 25°C three days post-hatching, was significantly higher in animals from cooler incubation temperatures. Temperature preference was recorded in thermal gradients for eight weeks post-hatching; turtles from cooler incubation treatments chose significantly warmer temperatures. These results suggest that cooler incubation temperatures trigger physiological and behavioral mechanisms that increase energy turnover. Thyroid hormones influence energy use in many animals. I measured blood thyroxine (T4) levels of 3 day old turtles; turtles from the 21.5°C incubation had significantly higher blood T4. Additionally, exogenous triiodothyronine, applied to the eggshell at mid-incubation, mimicked the effects of low incubation temperature on hatchling energy use. Thyroid hormones may mediate egg temperature effects on turtle energy use. These studies were controlled for egg temperature effects on turtle sex, and the results have implications for the evolution of environmental sex determination.

43.8

WATER METABOLISM OF ALASKAN SLED DOGS. Kenneth W. Hinchcliff, Gregory A. Reinhart*, John R. Burr*, and Richard A. Swenson*. College of Veterinary Medicine, The Ohio State University, Columbus, OH and * Research and Development, The Iams Company, Lewisburg, OH.

Alaskan sled dogs have metabolizable energy intakes in excess of 4100 $kJ/kg^{0.75}/d$ (47,000 kJ/dog/d) during long distance sled dog races (FASEB J 1994:8(5);A791). The high metabolizable energy intake of these dogs mandates a similarly high potential renal solute load. We measured water turnover and factors influencing urine volume and composition in 2 groups of highly trained Alaskan sled dogs. One group of 12 dogs (EG) ran in a 490 km sled dog race while a second group of 6 dogs (SG) were housed in unheated kennels. Body water turnover was estimated using deuterium oxide. Simultaneous urine and blood samples were collected before, at the midpoint, and immediately after the race. Average ambient temperature was -32 C (range -23 to -40C). EG and SG dogs weighed 26.9 +/- 0.85 kg and 22.5 +/- 1.5 kg, with total body water of 0.71 +/- 0.02 l/kg and 0.68 +/- 0.03 l/kg, respectively. Average water turnover of EG and SG dogs was 5.03 +/- 0.59 and 0.91 +/- 0.1 l/d. Serum [Na] and [K] were significantly different between groups during the race. There were significant differences ($P < 0.05$) between groups in plasma renin activity, and plasma aldosterone, atrial natriuretic peptide, and vasopressin concentrations. Similarly, EG dogs had significantly different fractional excretion of Na, K, Cl, and osmoles during the race. These data demonstrate that the high water turnover of Alaskan sled dogs during prolonged exercise is associated with significant changes in serum electrolyte concentrations and tubular reabsorption of Na, K, and Cl, the latter mediated by changes in plasma aldosterone and vasopressin concentrations.

43.9

EVAPORATIVE WATER LOSS IN NINE INSULAR LIZARD POPULATIONS OF THE GROUP *ANOLIS CRISTATELLUS* IN THE BRITISH VIRGIN ISLANDS. Razi Dmi'el, Gad Perry*, and James Lazell*. 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 (Rs) in eight insular populations of the lizard *Anolis cristatellus* and in one population of *Anolis ernestwilliamsi* 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 s cm^{-1}). 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 O_2 consumption; VO_{2max}) 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 VO_{2max} (especially during ontogeny), or the effects of routinely encountered parasitic infections on VO_{2max}. We examined VO_{2max} (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 VO_{2max} and body mass in 28-day old chicks but had no measurable effect in adults. Males had significantly higher VO_{2max} than females in both adults and chicks. The VO_{2max} of adults was highly repeatable (r=0.5 - 0.91; P <.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 BASAL RATES OF OXYGEN CONSUMPTION IN HOUSE MICE. Michael R. Dohm*, Jack P. Hayes*, and Theodore Garland, Jr. Dept. of Zoology, University of Wisconsin, Madison, WI 53706

We investigated the quantitative genetic basis of maximal locomotor performance and activity metabolism using a genetically variable, randombred strain of laboratory mice as a model system. We used a combined parent-offspring, half-sib, full-sib breeding design, with crossfostering, to estimate narrow-sense heritabilities (h_n^2 = additive genetic variance/total phenotypic variance) and genetic correlations. Prior 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 higher heritability (h_n^2 = .30, chi^2 = 6.07, df = 1, P < 0.05) than did forced maximal sprint running speed (h_n^2 = .14 for trial 1, chi^2 = 2.14, P > 0.05). Phenotypic correlations between speed and endurance were low (r_P = 0.017, P = 0.80), but the additive genetic correlation (r_A) was large, negative, and statistically significant, suggesting a necessary trade-off. Both residual maximal oxygen consumption (VO_2max) and basal metabolic rate (BMR) showed low heritability. VO_2max and BMR were uncorrelated phenotypically (r_P = +0.058, P = 0.29), but r_A 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 IBN-9111185 and IBN-9157268 to TG.

43.12

IS LOCOMOTION MORE COSTLY IN THE COLD RELATIVE TO INACTIVITY? Eileen Zerba, Ali Dana* and Matthew Lucia*. Colgate University Biology Department, Hamilton, NY 13346

Endothermic animals active at cold ambient temperatures must allocate energy to meet both thermostatic 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 respirometry system. The average specific metabolic rate for resting birds exposed to wind was 154.2 $\pm$ 20.5 ml of O_2/hr and that of exercising birds was 157.3 $\pm$11.5 ml of O_2/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. IBN-9306571.

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 boreoarctic 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-5,8,14,17-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* do not release EHP into the seawater, other than at the time of naupliar liberation. Secondly, barnacles fed liposomes enriched with EPA do not liberate their nauplii. Finally, barnacles fed on liposomes containing ^{14}C-EPA do not excrete radiolabelled EHP. Based on these results, it now seems likely that, in accord with the general scheme of eicosanoid biosynthesis, precursor fatty acid is released from membrane lipid stores. Preliminary results, obtained both *in vitro* and *in vivo* with inhibitors of lipoxygenases (LPO's), indicate that EPA 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.

Supported by the Natural Environment Research Council of the UK.

43.14

SNAPPING TURTLE EGGS THAT GAINED MASS DURING INCUBATION HAVE MORE ALLANTOIC 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 29°C usually absorb water during incubation. Though others have shown that water content of yolk was higher but water content of embryos 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 24.5°C in wet (0.05-0.07 gm water/gm dry sand; ~ -5 kPa) or dry (0.01-0.02 gm water/gm dry sand;~ -30 kPa) sand in 5 gal. plastic buckets. Groups of eggs were weighed and opened on days 33, 45, 54 or 63 of incubation to determine fluid volume and ionic composition of allantoic and amniotic fluid compartments. Yolk water content was also measured in both groups. Wet sand eggs (WSE) gained an avg. of 6.6 gm (= absorbed water) during incubation while mostdry sand eggs (DSE) only maintained initial egg mass. If any DSE began to lose mass, embryonic death occurred soon afterward. Amniotic fluid volume, total osmolarity and [Cl ion] were not different between groups. Embryos from WSE had significantly larger yolk water content and allantoic fluid volume than DSE embryos. Total osmolarity and [Cl ion] 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 Science.

43.15

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. Hatchling mass was also influenced by initial egg mass and clutch of origin. Hatchlings were kept individually at either 26 or 29 C, fed identical rations, and weighed biweekly for 8 months. Turtles grew to different sizes. The most important predictor of turtle mass after 8 months of growth was initial hatchling mass. Important secondary predictors of 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 hatchling mass.

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43.16

COMPARATIVE EMBRYONIC DEVELOPMENT IN CHICKENS WITH DIVERGENT PATTERNS OF POSTNATAL GROWTH. Nancy J. Clum and Deedra M. McClearn. Cornell University, Ithaca, N.Y. 14853.

Growth does not begin at hatching, but is a continuation of processes begun *in ovo*. Because cell number of some tissues is fixed by the time of hatching, postnatal growth potential may be determined by patterns of embryonic development. Classical staging techniques and morphological measurements of bone, cartilage, feather and intestines were used to test the hypothesis that differences in postnatal growth would be reflected in patterns of embryonic tissue partitioning. During the second quarter of incubation, the line with higher postnatal growth staged significantly earlier (i.e., was less developed) than the line with lower postnatal growth. By the third quarter of incubation, both lines staged equally. Not all tissues appeared to "catch-up" at the same rate. Bone growth and ossification of wing bones did not generally differ between lines, but in the leg bones the initial degree of ossification was lower, and subsequent rate of ossification higher in the line with high postnatal growth. There were no differences in gut development between the two lines, but the line with higher postnatal growth had less feather development than the line with lower postnatal growth. These results indicate that tradeoffs in resource allocation occur both between and within tissues during embryonic development in relation to selection for increased postnatal growth rate potential.

MUSCLE AND LOCOMOTOR ADAPTATION

44.1

SIGNIFICANCE OF POLYPLOIDY IN THE THERMAL ACCLIMATION OF C-START PERFORMANCE IN FISH: AN INTEGRATED STUDY OF MOLECULAR AND CELLULAR PHYSIOLOGY AND ORGANISMAL PERFORMANCE. Timothy P. Johnson* and Albert F. Bennett. Dept. of Ecology and Evolutionary Biology, University of California, Irvine, CA92717.

Thermal acclimatory responses of fast twitch muscle fibers have been clearly demonstrated for cyprinid fishes. However, many studies have failed to document similar findings in other groups of fish, reptiles and amphibians. It has been suggested that this apparently unique ability of cyprinid muscle to adapt to prolonged temperature exposure is related to their polyploid genetic structure. In this study we therefore compared the acclimatory ability of two teleost species, a polyploid known to acclimate (goldfish, *Carassius auratus*), the other a diploid (killifish, *Fundulus heteroclitus*) previously shown to lack thermal acclimatory ability. Thermal acclimatory responses of C-start (escape) swimming and associated muscle function were measured in animals acclimated to 10, 20 and 35°C for four weeks.

The activity of fast muscle myofibrillar ATPase measured at 10°C, increased by 687% ($P<0.0001$) in goldfish and only 55% ($P<0.002$) in killifish, following a period of acclimation from 35 to 10°C. This increase in activity was accompanied by changes in the expression of myosin heavy chain isoforms in goldfish only. Fast muscle twitch contraction kinetics also increased following cold-acclimation by 50% ($P<0.0001$) in the goldfish and only 25% ($P<0.0002$) in the killifish. These findings were mirrored by changes in organismal performance (maximum velocity, maximum angular velocity and distance moved in 40ms). All kinematic variables measured at 10°C were highly significantly affected by acclimation temperature in goldfish ($P<0.0001$), whereas only angular velocity was significantly correlated with acclimation temperature in the killifish ($P<0.01$). The magnitude of the acclimation response for all experimental parameters was highly significantly different in the two species ($P<0.0001$, Tukey's jack-knife method). The results support the hypothesis that polyploidy is a significant factor in the evolution of thermal acclimatory ability in cyprinid fishes.

44.2

EVOLUTIONARY RESPONSE OF SWIMMING PERFORMANCE IN GUPPIES TO DIFFERING PREDATION INTENSITIES Alistair J. Cullum. U.C. Irvine, Irvine, CA 92717

Locomotor ability has often been perceived as important in the outcome of certain types of predator-prey interactions. 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 (or "C") start, a hard-wired, turn-and-"kick" escape response to approaching shock waves, while 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 ("C") starts have not shown any evolutionary divergence between the two sorts of populations, but that subsequent burst swimming is faster in high-predation populations. This work was supported in part by a National Science Foundation Graduate Fellowship.

44.3

CLONING AND CHARACTERIZATION OF THE RYANODINE RECEPTOR α ISOFORM FROM FISH. Jens P.C. Franck*, Mark S. Beamsley*, John E. Keen, Richard L. Londraville, and Barbara A. Block. Stanford University, Hopkins Marine Station, Pacific Grove, Ca. 93950.

Ryanodine receptors (RYR) are intracellular channels that release Ca^{2+} from internal stores and are enriched in the sarcoplasmic reticulum of striated muscles. The primary sequence of three mammalian RYR isoforms have been deduced from cloning and sequencing of cDNAs from skeletal (RYR 1), cardiac (RYR 2) and brain (RYR 3) tissues. While mammalian skeletal muscles predominantly express RYR1, most non-mammals express two skeletal muscle isoforms, α and β. An exception to the non-mammalian condition is the fast contracting superior rectus muscle of blue marlin which predominantly expresses the α RYR isoform. Using both PCR generated probes and RYR antibodies we have isolated clones from a superior rectus muscle library representing approximately 70% of the complete marlin α cDNA sequence. RT-PCR from total RNA with specific primer pairs detected the α RYR isoform in superior rectus and white muscle tissues. Phylogenetic analyses of the derived amino acid sequence indicates homology with the mammalian α RYR isoform. A multiple alignment of the derived amino acid sequence from the marlin α RYR cDNA with the three rabbit RYR isoforms (skeletal, cardiac, brain), human skeletal, and frog α and β isoforms was generated using the *Drosophila* RYR sequence as a designated outgroup. The marlin α RYR clustered in the same clade as the frog α and rabbit and human skeletal sequences, distinct from the clade clustering the frog β and mammalian brain isoforms. The marlin α RYR cDNA sequence therefore appears to code for a skeletal-like (RYR1) isoform of the RYR gene family. (Research supported by NIH)

44.4

PHYSIOLOGICAL DIFFERENCES BETWEEN THE α AND β RYANODINE RECEPTORS OF FISH SKELETAL MUSCLE. John O'Brien[1], Hector H. Valdivia[2] and Barbara A. Block[3]. [1]U. of Chicago, [2]U. of Wisconsin, and [3]Stanford University.

Calcium release from intracellular stores is mediated by ryanodine receptors (RYRs) in a wide variety of excitable and non-excitable cells. In mammalian striated muscle, RYRs occur in at least two forms: a skeletal isoform that is expressed in fast- and slow-twitch skeletal muscle and a cardiac isoform expressed in heart. Despite extensive sequence homology between the two RYRs, there are distinct physiological differences between them. In fish, as in most non-mammals, the majority of skeletal muscles express two RYR isoforms (α and β) together while certain fast-contracting muscles express only α. The functional implications of this expression pattern are unknown but are useful for studying physiological differences between isoforms. Using [^{3}H] ryanodine binding as an indicator of channel activity, and reconstituting Ca^{2+} release channels into planar lipid bilayers, we identified differences in the Ca^{2+}-dependence of channel activity of the α and β isoforms from fish. In extraocular muscles of marlin and toadfish swimbladder muscles, which express the α isoform alone, [^{3}H] ryanodine binding has a threshold for activation at 100 nM Ca^{2+}, peaks at 10 μM Ca^{2+} and inactivates at 3 mM Ca^{2+}. This biphasic response results in a bell-shaped curve similar to that of rabbit skeletal muscle. Fish cardiac RYR displays a similar activation threshold but shows optimal [^{3}H] ryanodine binding at 100 μM Ca^{2+} and only 20% inactivation at 3 mM Ca^{2+}. In swimming muscle, which expresses both α and β isoforms, the Ca^{2+}-dependence of binding is a blend of cardiac and skeletal features. Tetracaine (100μM) preferentially inactivates the α RYR, and only partially inactivates swimming muscle ryanodine binding indicative of inactivation of α and not the β isoform. Single channel recordings from swimming muscle SR also distinguish two different calcium channels based on conductance properties and calcium dependence of channel opening. The possible roles of the physiological differences between RYR isoforms are discussed.

44.5

HIGH FAT DIET IMPROVES AEROBIC PERFORMANCE BY BUILDING MITOCHONDRIA. C. Richard Taylor, Hans Hoppeler, C. Kennedy, T. Valenski, T. J. Roberts and P. Weyand. CFS, Harvard University, Old Causeway Rd, Bedford, MA, 01730 and Dept. of Anatomy, Univ. Berne, Berne, Switzerland.

Eating a high fat diet increases maximal rates of fat oxidation in exercising humans, pigs and rats. We have found that during exercise most of the fat is supplied from fat droplets within the muscles cells. Every fat droplet is in direct contact with a mitochondria, suggesting that the fatty acids are released directly from the droplet into the mitochondria for ß 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 fat oxidation of trained dogs fed a normal commercial diet (25% of the calories from fat). Then we fed the dogs a high fat diet containing 65% of the calories from fat* while holding their training constant. Maximal rate of fat oxidation increased dramatically on this diet, reaching 3343 µmol O_2/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 7983 µmol O_2/min•kg (± 446 SE), also an increase of about 50%. Mitochondiral 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 ANAEROBIC ENZYME ACTIVITY. In-Ho Choi and Kyoungsook Park*. Yonsei Univ., Wonju, Republic of Korea 222-701.

A correlation between variability 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 ($m.sec^{-1}$) 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 ± 0.12SD, 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 (ranging 189 - 272 $mN.mm^{-2}$) was about the same among the three species and did not correlate with jump velocity. Relative anaerobic capacity (lactate dehydrogenase activity/citrate synthase activity) of *R. nigromaculata* was significantly greater than that of *R. rugosa* and *B. orientalis*, while it was nearly the same between *R. rugosa* 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 KOSEF Grant 941-0500-048-2 and Yonsei University Faculty Grant 1992 to Choi.

44.7

Muscle length and relaxation time in working frog muscle. E.Don Stevens. Zoology, Univ of Guelph, Canada N1G 2W1. (supported by NSERC)

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 report similarly) that 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 strains from 0.06 to 0.12. At very small strains (i.e., about 15 to 30 nm/halfsarc) 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.

44.8

THE LONG AND SHORT OF IT: HOW MUCH DO MUSCLES LENGTHEN DURING RUNNING? R.L. Marsh, T.J. Roberts, C.I. Buchanan*, P.G. Weyand, and C.R. Taylor. Biology, Northeastern University, Boston, MA 02115 and CFS, 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. Cine 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 stretches, 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 (L_0). 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 up to 3.5 m/s, a fast run for the turkey. At speeds over 2.0 m/s the muscle fascicles did stretch, but this stretch was less than 3% of L_0. In addition to this confirmation of our initial hypothesis, we also discovered that during peak force production the fascicles were operating on the 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. Supported by NIH grants AR39318 to R.L. Marsh and AR18140 to C.R. Taylor

44.9

DOES STEP LENGTH DETERMINE THE COST OF WALKING IN QUADRUPEDS? P.G. Weyand, C.M. Stoffel, J.E. Bildorff, A.M. Cheng, T.J. Roberts, C. R.Taylor. C.F.S., Museum of Comparative Zoology, Harvard University, Bedford, Mass. 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 center of mass moves while the foot is in contact with the ground (step length, Lc). The longer running steps of large 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. We undertook 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 flow system 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 (168 kg). We found that for running COT was constant across speed, but for walking COT was minimized at a moderate speed (preferred speed, duty factor 0.65). In large dogs and goats the 20% longer Lc's during walking 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 gaits, COT did not differ between walking at the preferred speed and running. We conclude that during quadrupedal walking at the preferred speed, as during running, that energetic cost is directly related to step length. This work was supported by NIH Grant R01AR18140

44.10

ACCLIMATION TO WEIGHTLESSNESS. Robert W. Phillips, Frank M. Sulzman and Joan Vernikos*. Dept Physiology Colorado State U. Fort Collins, CO 80523, Life and Biomedical Sci Div. NASA Headquarters, Washington DC 20546

To date no organisms have been exposed to the microgravity of space for a sufficient period to induce true 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 their response was very similar to 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 Flight animals videotaped 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

OXIDATIVE CAPACITY AND MUSCLE MASS RESPONSES TO 14-DAY HINDLIMB SUSPENSION IN A HIBERNATOR. Sandra Khatchadourian*, F. Otis Stephen*, Steven J. Wickler and Donald F. Hoyt, California State Polytechnic University, Pomona,CA 91768

Hindlimb suspension studies are used for modeling muscle disuse in non-hibernators such as rats. Suspension produces muscle atrophy and decreases in oxidative capacity. During normothermic detraining the muscles 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 these squirrels. Animals (collected August ,1993) 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 cages. Masses of the gastrocnemius/plantaris (G/P), soleus (SOL), and extensor digitorum longus (EDL) were measured. Citrate synthase (CS), an indicator of oxidative capacity, and HOAD (an indicator of fat oxidation) were measured. Suspension produced muscle atrophy in G/P (23%), EDL (23%), and SOL (16%); and decreases 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. HOAD activity per unit mass 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 GMGS may be due to seasonal changes. Our previous detraining studies were conducted in August and September, while our suspension study was conducted in late October. We are currently planning a similar suspension experiment in late August-early September. Supported by an NIH grant to DFH and SJW, RAP 1 R15 AR-39893-01A2.

44.12

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

The purpose of this study was two-fold: (1) to compare immunohistochemical techniques for fiber typing with standard qualitative cytochemical techniques on ground squirrel muscle; and (2) assess the 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. The plantaris was removed, stretched to resting length, and frozen for histochemistry. The remaining animals were housed in rodent cages with food and water *ad libitum* for one month of detraining and then sampled. Frozen muscle was cut in 8 μm serial sections. One section was stained using MY-32 anti-fast HMC for staining of fast fibers (Sigma™ Chemical). The second section was stained for myosin ATPase using standard histochemical techniques employing an alkaline (pH=9.75) pre-incubation to stain fast (type II) fibers. Thirty-forty fibers from each serial section for six animals (3 controls, 3 treatments) were compared for staining characteristics using the two techniques. Only one fiber analyzed was not typed the same by both techniques. The plantaris consists of 80% fast, 20% slow fibers and there was no difference between control and treatment animals. For analysis of the effect of treatment on fiber areas, ANOVA for repeated measures was used to analyze 100 fast (dark staining) and 100 slow (light staining) fibers from each individual. There was an 18% atrophy in the fast fibers of detrained animals (1872±17 vs. 1544±13 μm^2, ±1 SE). Slow fibers atrophied 4% (1824±18 vs. 1750±14 μm^2). Reducing activity in wild squirrels produces atrophy of both fast and slow fibers. (Supported by an NIH grant to DFH and SJW, RAP 1 R15 AR39893-01A2).

44.13

HINDLIMB SUSPENSION AND MUSCLE FIBER AREAS IN THE GOLDEN MANTLED GROUND SQUIRREL (*Spermophilus lateralis*). C. S. J. Tseng*, T. P. Nguyen*, and S. Yoshizaki*, S. J. Wickler, and D. F. Hoyt, California State Polytechnic University, Pomona, CA 91768

One model of muscle disuse is hindlimb suspension which produces both hypokinesia and hypodynamia. In rats, this produces greater atrophy of slow fibers than fast fibers. Our laboratory has an interest in disuse atrophy in species that naturally undergo periods of disuse, namely, hibernators. In the current study, we examined the response of locomotor muscles in ground squirrels undergoing hindlimb suspension. Ground squirrels (n=20) were captured in the Sierra Nevada in late July. After 3 months in the laboratory, one half of the animals were placed into a jacketing device that prevented the hindlimbs from touching the cage floor. After 14 days, hindlimb musculature was removed, stretched to resting length, and then frozen in isopentane, cooled in liquid nitrogen. Muscles studied were the plantaris, soleus and extensor digitorum longus (EDL). 8 μ sections were cut and stained for myosin ATPase using a pH 9.75 preincubation. Fibers were identified as either fast (dark staining, type II) or slow (light staining, type I).

	Plantaris		EDL	Soleus
% Fast	81		100	0
Areas (μm^2)	Fast	Slow	Fast	Slow
Control	1590±31	1215±22	1780±29	1251±22
Suspension	1194±35	1051±27	1085±20	853±18
Sig. Diff.	*	ns	*	*

Fourteen days of hindlimb suspension produced significant atrophy in all muscles. Both fast and slow fibers atrophied. Supported by an NIH grant to DFH and SJW (RAP 1 R15 AR-39893-01A2).

44.14

DISUSE ATROPHY AND OXIDATIVE CAPACITY IN ANTELOPE GROUND SQUIRRELS, A NON-HIBERNATOR (*Ammospermophilus leucurus*). Steven K. Teh, Thao P. Nguyen*, Steven J. Wickler and Donald F. Hoyt, California State Polytechnic University, Pomona, Ca. 91768

Hibernating ground squirrels respond to reduced activity with muscle atrophy and increased oxidative capacity (as assessed by citrate synthase activity). However, more traditional models, including the laboratory rat, show muscle atrophy and decreased oxidative capacity. The present study reports the responses of a phylogenetically more closely related non-hibernating species, the Antelope Ground Squirrel (AGS). AGS were captured in mid-September 1993. Controls (n=10) were sampled immediately for carcass mass (body mass minus ski and, viscera), and muscle mass of the gastrocnemius/plantaris (G/P), extensor digitorum longus (EDL) and soleus. Experimentals (n=13) were housed in rodent cages with food and water *ad libitum*, for two months and then sampled as above. No differences were found in body and carcass masses between controls and experimentals. The G/P and soleus atrophied in experimental animals (20% and 11% respectively). The change in the EDL was not significant (p=0.08). In addition, CS activity was not different for G/P (23.4±1.3 vs 25.1±.9 U/g for controls and experimentals, respectively) nor soleus (35.3±1.3 vs 37.7±1.6 U/g). CS activity was increased in the EDL (20.6±1.3 vs 26.9±1.0 U/g). 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 profused isolated heart of AGS respond similiarly to the hearts of hibernators. Supported by a NIH grant to DFH & SJW (RAP 1 R15 AR-39893-01A2).

44.15

Triiodothyronine (T_3) and Insulin Concentrations Associated with Two Months Normothermic Detraining in the Golden-Mantled Ground Squirrel (*Spermophilus lateralis*). **H. Taren Tseng*, Christine S. J. Tseng*, Donald F. Hoyt, & 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 Journal* 8: A574, 1994). Because the latter study was conducted at the time of year when the 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 that observed in rats under similar experimental conditions. The present study was undertaken to test the hypothesis that this unusual response to normothermic detraining in GMGS may be due to hormonal mechanisms involving triiodothyronine and insulin. Twelve squirrels were captured in late July of 1993 and housed individually in lab cages (46L x 28W x 20H cm) with food 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 and assayed for concentrations of free T_3 and insulin using radioimmunoassay. Data were analyzed by ANOVA with repeated measures. Both T_3 and insulin levels increased during this period (T_3: 0.518 ± .145 to 1.09 ± .20 pg/ml, p=0.045; Insulin: 8.75 ± 1.7 to 16.2 ± 4.1 μIU/ml, p=0.046). These elevated levels suggest that T_3 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 (RAP1R15AR3989301A2).

44.16

KINEMATIC ADJUSTMENTS OF AN INTERTIDAL CRAB LOCOMOTING 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 make predictions about the mechanics of aquatic versus terrestrial pedestrian locomotion. We determined 3-D kinematics of 34 points during one stride of the intertidal crab (Grapsus grapsus) as it moved over a flat substratum through air and water. Air and water trials at matched average velocities (10 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 and contact time was 1/2-1/3 that in air. Although the height of the body was not different, the width of the crab's stance was 19% greater in water than air. This more stable stance still resulted in more pitching and rolling in water than in air. The angle swept by the merus-carpus joint was also smaller under water, more so in the trailing legs. As predicted a greater degree of freedom in gait use was found for aquatic movement. Locomotion in water showed much more variability in contralateral leg kinematics. In water, animals often cycled adjacent legs or leading and trailing legs of the same pair at different rates, with some legs not cycling at all throughout the stride. Studying locomotion in air versus water in amphibious animals such as crabs can to lead to the integration of terrestrial locomotor dynamics with hydrodynamics, as well as to ideas on the evolution of terrestriality.

44.17

MULTIPLE MUSCLE KINEMATIC SIMULATION OF RUNNING ROACHES. Anna N. Ahn* and Robert J. Full. Univ. of California, Berkeley 94720

In an effort to address the complexity of multiple muscle systems during terrestrial locomotion, we simulated a stride 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, origin and insertion positions 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 maximum during only the first third of the swing phase when inertia of the leg is important. Major extensors (177a, 177c) and flexors (181a, 181b) operated on the plateaus of their force-length curves (>86% max isometric force, F_o), whereas the smaller muscles did not (177e,177d, 182d; 44 - 100% F_o). Because cockroach musculo-apodeme actuators are considered to be stiff (0.07-0.53; apodeme slack length to optimal muscle fiber ratio), we were able to estimate muscle fiber velocities. Extensors maintained a constant velocity (2-6 lengths/sec) during the stance phase. Flexor velocities (4-16 lengths/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 PYI DCB 90-58138.

44.18

MECHANICAL POWER OUTPUT OF THE MUSCULAR SYSTEM AND MAXIMUM RUNNING SPEED. D.T. Moran*, C.T. Farley, and M.G. Emshwiller*. Dept. of Human Biodynamics, University of California, Berkeley, CA. 94720-4480.

We tested the hypothesis that running 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 angle of the running surface (0°, 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. = 25°C). Force platform analysis revealed that the mechanical power output of the center of mass ($P_{C.O.M.}$) during sprinting on level ground was approximately 1 W • kg^{-1}. For both species, $P_{C.O.M.}$ was dominated by the horizontal (anterior-posterior) component which was approximately 10 times greater than the vertical component and 100 times greater than the lateral component. When the lizards ran at maximum speed up a 40° incline, the mechanical power required to lift the center of mass up the hill was approximately 4-fold greater than $P_{C.O.M.}$ 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 muscular system to deliver mechanical power does not limit maximum speed. Supported by NIH AR08189.

44.19

ENERGETIC COST OF LOCOMOTION IN HORSES: EFFECTS OF SPEED, GRADE, CARRYING AND PULLING WEIGHTS. B. L. Smith*, J. H. Jones, N. L. Couper* and C. Garlow-Hatch*. University of California, Davis, CA 95616.

Quadrupeds use their muscles in different ways during cursorial locomotion, *e.g.*, for doing positive or negative work, or generating force with no length change. To determine the effects of using muscles in different ways on the energetic cost of locomotion, we measured oxygen consumption ($\dot{V}O_2$) and lactate accumulation rates as five 500 kg Thoroughbred horses ran on a treadmill at different speeds and grades while carrying weights on their backs or pulling horizontally against weights suspended from a pulley and attached to a harness. In two series of experiments horses ran at speeds of 1.5 m/s (walk), 4 m/s (trot), 7 m/s (canter) and 10.5 m/s (gallop), or at speeds of 1.5 m/s, 3 m/s (trot), 4.5 m/s (trot) and 6 m/s (canter). Slower speeds minimized lactate accumulation and caused metabolic power to be more fully aerobic. Horses ran up grades of 0%, 3%, 6%, 9% or 12% while carrying weights in a backpack saddle or connected via a pulley and harness to weights equal to 0%, 5%, 10% or 15% of body mass (M_b). Maximal $\dot{V}O_2$ were identical for individual horses no matter what combination of speed, grade and carried or pulled weight was used. For all grade and weight combinations, $\dot{V}O_2$ increased linearly with speed. The slopes of the $\dot{V}O_2$ *vs.* speed relationship were similar at different grades, with steeper grades having higher intercepts. Pulling a given fraction of M_b required more energy than carrying the same mass. Energetic cost of carrying or pulling weights increased with a slope similar to that for increasing speed up a grade. Carrying or pulling greater weight increased the intercept of the $\dot{V}O_2$ *vs.* speed relationship. Supported by the UCD Equine Research Laboratory with contributions from the Oak Tree Racing Association, California Satellite Wagering Fund, and private donations.

44.20

ADAPTATION TO PHYSICAL WORKLOAD IN ELDERLY SPORTSMEN Georgiy V. Korobeiynikov Institute of Gerontology, Kiev, 254114,Ukraine

The study involving two groups (group 1 - 30 sportsmens aged 18-28; group 2 - 16 sportsmens aged 42-55, respectively). Was undertaken to assess the physiological adaptation of hearth rate in response to physical workload.

The physical load increasing two step-wice: 50 Watt and 100 Watt, during 6 min.

The following parameters were registered: cardiac rhythm, arterial blood pressure, muscle force, muscle endurance, physical working capacity (PWC) and functional reserves of hearth (FRH).

The data showed, that the 42 % of young sportsmens and 20 % of elderly sportsmens has high level of the PWC. At the same time, 47 % of young sportsmens and 40 % of elderly sportsmens has high level of the FRH. This demonstrate an age-relation decrease of the physical working capacity with conservational of the functional reserves in the elderly sportsmens. In other words, the muscle force and endurance decrease progressively with increasing age.

The adaptation on physical workload in elderly sportsmens characterized of the mobilization of functional reserves of the heart.

54.1

IS LACTATIONAL PERFORMANCE LIMITED BY THE ABILITY TO EAT ENOUGH FOOD, OR THE ABILITY TO MAKE ENOUGH MILK? Kimberly A. Hammond and Jared Diamond, UCLA, Los Angeles, Ca. 90024.

Is metabolic performance limited by an animal's physiological capacity to eat and digest a sufficient amount of food, by the capacity of energy output organs to expend the energy gained from that food, or perhaps by a limit residing in shared machinery beyond the intestine (e.g., liver or kidneys)? We attempted to answer these questions by using lactation in mice as a model of a situation in which energy demand is at its highest for the mother mouse. As litter size increases in lactating mice, the mother's food intake increases, but individual pup mass decreases. This suggests that there is a limit to lactational, and hence metabolic, performance. To address this problem, we increased energy demands in lactating females by forcing them to raise their litters at cold temperatures (5°C). In this manipulation, daily food intake of the cold/lactation mothers increased by 3.9 g (compared to intake at 23°C), regardless of litter size. The lactating mother's intestinal capacity to transport nutrients at 5°C also increased over that of mothers at 23°C. Individual pup mass, however, did not differ between litters at 5°C and 23°C. Thus, neither food intake nor gut capacity is limiting to lactational performance and hence, to metabolic output. Finally, using a surgical technique to remove half of the mammary glands, we tested the hypothesis that the capacity to produce milk limits lactational performance and results in lower pup masses at larger litter sizes. From this manipulation we have determined that at the largest litter sizes, and hence the highest energy demand, lactation is limited by the capacity to produce enough high-quality milk. At the smallest litter sizes, lactational performance is limited by the cost to upregulate the gut and mammary glands.

54.2

QUALITATIVE AND QUANTITATIVE STRATEGIES OF THERMAL ADAPTATION OF GRASS CARP (*CTENOPHARYNGODON IDELLA*) CYTOPLASMIC MALATE DEHYDROGENASES.

Jen-jen Lin, Shirley MacLeod*, Ing-Nan Chen*, and Ching-ming Kuo*
National Taiwan Univ., Institute of Fisheries Science, Taipei, Taiwan 10764, ROC

Cytoplasmic malate dehydrogenase (cMDH) is involved in gluconeogenesis, lipogenesis and malate-aspartate 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 encoding the thermostable and thermolabile cMDHs. Structural and kinetic characteristics of the two isozymes partially purified from white muscle showed that the half life of thermostable cMDH at 42°C is two order higher than that of the thermolabile form. The thermostable cMDH was more sensitive to the inhibition of malate, while the thermolabile cMDH 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 cMDH was more than twice that of the thermostable cMDH. In contrast, the Km value of OAA for the thermolabile cMDH was about one third that of the thermostable cMDH. Furthermore, temperature acclimation of grass carp showed that white muscle thermolabile cMDH appeared in higher concentrations in white muscle of 11°C-acclimated fish, while the thermostable 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 cMDH isozymes.

54.3

THE ENERGETICALLY OPTIMUM PATH IN GRADIENT LOCOMOTION
Alberto E. Minetti
Dept. of Muscle Physiology - Istituto Tecnologie Biomediche Avanzate - C.N.R. Via Ampére 56, 20131 Milano, Italy

Fiftysix years ago R. Margaria extensively investigated the energy cost of walking and running .at gradients ranging -40 to +40% (Margaria, 1938 Atti Accademia Nazionale dei Lincei 7: 299-368). His graphs show that the cost of running (CR), at whichever speed, and of walking (CW), at the optimum speeds, are minimum at a gradient of about -10% (downhill). CR and CW were expressed per unit distance. When we have to move in the mountains the departure and the arrival locations are given, and one possible challenge is to choose the path (gradient) minimizing the overall energy expenditure. In uphill locomotion, for example, the steepest path is associated with the highest metabolic cost (per unit distance) and with the lowest distance (d) to be covered, the reverse being true for the shallower positive gradients. This suggest the existence of optimum paths (or gradients) for uphill and downhill locomotion. Considering that a change in altitude of Δh corresponds to a distance to be walked or runned equal to $\Delta h/(\sin(\arctan(i))$ (or $\approx \Delta h/i$), where i is the chosen gradient, the multiplication of CR and CW by d (CRpath and CWpath, respectively) leads to the following results: a) the optimum path for walking uphill occurs at a gradient of about +28 % and at a speed of about 2 km/h, b) the optimum path for walking downhill occurs at a gradient of about -25 % at a speed of about 4-5 km/h, c) CRpath, which is speed independent, decreases with increasing gradient but does not show a minimum within the investigated range, suggesting that the best strategy to minimize the metabolic energy spent is to run at the steepest (uphill or downhill) gradient and at the most comfortable speed. The flatness of CWpath vs. gradient curve, though, suggests that for energy-saving purposes gradients from +15% to +30% (uphill) and from -40% to -15% (downhill) should be chosen in walking.

54.4

EVIDENCE AGAINST A DISCRETE PEAK FOR AN OPTIMUM HEMATOCRIT FOR MAXIMUM OXYGEN CONSUMPTION (Vo_{2max}) AND FOR MAXIMUM PROLONGED SWIMMING (U_{crit}) IN RAINBOW TROUT. Anthony P. Farrell and Patricia E. Gallaugher, Simon Fraser University, Burnaby, B. C., V5A 1S6, Canada.

This study challenges the idea that a discrete optimum hematocrit (Hct) exists and that this represents a trade off between arterial O_2 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, normocythemic and polycythemic trout at 13°C while measuring various cardiorespiratory variables, including U_{crit}, Vo_{2max}, and arterial O_2 transport (To_2). A Hct of 27% was found to be normocythemic, but Hct varied between 23% and 33% for individual fish. Anemia (Hct < 22%) reduced To_2, U_{crit} and Vo_{2max}. Through the normocythemic and polycythemic range for Hct (23%-55%), To_2 was still proportional to Hct (as in anemic fish), but the gains in U_{crit} per increment in Hct were less pronounced. There was no peak value for U_{crit}. Furthermore, the peak Vo_{2max} occurred at a Hct of 42%, a value well above the normocythemic range. Similar relationships between U_{crit} and Vo_{2max} 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.

SUBZERO TEMPERATURE ADAPTATIONS OF POIKILOTHERMS

55.1

SEASONAL VARIATION IN THE CRYOBIOLOGY OF WOOD FROGS FROM PENNSYLVANIA. Jack R. Layne, Jr. Slippery Rock University, Slippery Rock, PA 16057.

Crystallization temperature, freeze tolerance, ice content, and plasma glucose were measured in wood frogs (R. 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 freezes lasting 2 wk and to -5°C but summer-collected frogs did not tolerate these conditions. The autumn-collected frogs had lower ice contents than summer-collected frogs. In all cases, the measured ice contents were substantially less than the expected ice contents. Plasma glucose and plasma osmolality were highest during the autumn (93 µmole/ml and 377 mOsmoles) when compared to levels during the spring (6.2 µmole/ml and 226.0 mOsmoles) and summer (2.7 µmole/ml and 194.5 mOsmoles). In conclusion, seasonal conditioning substantially influences several key aspects of the cryobiology of wood frogs.

55.2

COLD- AND DIET-INDUCED ACTIVATION OF Δ^9- DESATURASE IN CARP LIVER AND ISOLATED HEPATOCYTES. A.I.Macartney, P.Tiku and A.R. Cossins, Department of Environmental and Evolutionary Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK.

The most consistent cellular adaptation to cooling is 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 immunoassay and levels of desaturase mRNA by Northern analysis. A three day cooling regime imposed on 30°C-acclimated fish led to a 20-30 fold increase in activity and 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 prelimiary results indicate that desaturase induction is due, at least in part, to transcriptional activation. Levels of desaturase have also been measured in cultured hepatocytes from warm-acclimated carp. Culture at 30°C for up to 4 days led to 20-40 fold 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.)

ADAPTATIONS TO HIGH AND LOW OXYGEN STRESS

56.1

PROTEIN SYNTHESIS RATES DECREASE AFTER TWO HOURS OF ANOXIA IN ISOLATED PERFUSED TURTLE HEARTS. W. Driedzic and J. Bailey*, Mount Allison University, Sackville, New Brunswick, Canada, EOA 3CO

Protein synthesis, as measured by ^{3}H-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 PHE/mg protein.hr) than normoxia (0.36 nmole PHE/mg protein. hr). Mitochondrial protein synthesis rates decreased and the ratio of mitochondrial to total protein synthesis was lower after anoxic than normoxic perfusion. Citrate synthase activity also declined (normoxia-0.27; anoxia 0.12 µmole/g protein. min). Isolated 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 pHi. 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.S.E.R.C. of Canada.

56.2

Anoxia induced increase in cerebral blood flow in turtles: Role of adenosine

Patrick Hylland[1], Göran E. Nilsson[1] and Peter L. Lutz[2]
[1]Vertebrate Physiology and Behaviour Unit, Dept. Limnology, Uppsala University, Norbyvägen 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 0°C. In vertebrates from mammals to fish the brain is particularly sensitive to anoxia, rapidly losing its ATP levels. In order to maintain brain ATP, turtles utilize two cooperating strategies. An initial enhancement of glycolytic ATP production is follow by deep hypometabolism. An increased glycolytic rate would probably demand increased glucose delivery from the blood. Still there has been no studies on the time course of changes in turtle brain blood flow or underlying mechanisms. We have used epi-illumination microscopy to record blood flow velocity on the cortical surface of turtles. During anoxia the blood flow velocity increased x 1.7 after 45-75 min whereupon it fell back, reaching pre-anoxic values after 115 min of anoxia. Topical superfusing the brain with adenosine (50µM) during normoxia caused a 3.8-fold increase in flow velocity. Superfusing the brain with the adenosine blocker aminophylline (250µM) totally inhibited the effects of both anoxia and adenosine, while aminophylline had no effect on normoxic blood flow velocity. None of the treatments affected the systemic blood pressure. These results indicate an initial adenosine mediated increase in blood flow velocity during anoxia, probably representing an emergency response before deep metabolic depression sets in.

56.3

OUTFLOW OF K^+ IN TELENCEPHALON OF CRUCIAN CARP AND RAINBOW TROUT DURING ANOXIA: DO FISH HAVE ATP-SENSITIVE K^+ CHANNELS ?

Dan Johansson and Göran E. Nilsson, Vertebrate Physiology and Behaviour Unit, Department of Limnology, Uppsala University, Norbyvägen 20, S-752 36 Uppsala, SWEDEN

The crucian carp (*Carassius carassius*) is one of the most anoxia-tolerant vertebrates known. By using anaerobic glycolysis with ethanol as end product, the crucian carp is able to maintain ATP-levels and ion-homeostasis in the brain for long periods of anoxia. The rainbow trout (*Oncorhynchus mykiss*), on the other hand, is very sensitive to low oxygen availability and die rapidly in anoxia. In mammals, results have suggested that the ATP-sensitive K^+ channels (K_{ATP} channels) are of great importance for the early outflux of K^+ from neurons during anoxia. Thus, experiments on rat brain during anoxia have shown that extracellular K^+ levels ($[K^+]_o$) rise more slowly when these channels have been blocked by the K_{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^+ release during anoxia (rainbow trout) or during Na^+/K^+ pump blockade with ouabain (crucian carp). The results show that there is no difference in the rate of K^+ outflux between fish treated with glibenclamide and controls. This indicates that other mechanisms than K_{ATP} channels are responsible for the rise in $[K^+]_o$ in fish brain during energy deficiency or Na^+/K^+ pump inhibition.

56.4

REGULATION OF OXYGEN UPTAKE ACROSS TWO RESPIRATORY SURFACES IN THE MARINE WORM, *URECHIS CAUPO*.

Wendy E. Passman, David Julian and Alissa J. Arp. San Francisco State University, S.F., CA. 94132

The echiuran worm *Urechis caupo* inhabits intertidal mudflats along the central California coast where tidal cycles expose it to ambient PO_2's below 50 mmHg. To determine the mechanisms by which *U. caupo* maintains O_2 consumption in hypoxic water, we examined the regulation of O_2 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 PO_2's between 25 and 300 mmHg. At normoxia, over 60% of O_2 uptake occurs across the body wall, with the remainder occurring across the hindgut. *U. caupo* is a partial oxyregulator; as ambient PO_2 decreases from 150 (normoxia) to 25 mmHg, total O_2 consumption decreases by 50%. O_2 uptake across the body wall decreases by 60% over the same range, whereas hindgut O_2 uptake remains constant. Thus, as ambient PO_2 decreases, the average contribution of the hindgut to total O_2 consumption increases from 17% at 300 mmHg, to 36% at normoxia, and to 50% at hypoxia. This increase in O_2 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 O_2 uptake across the body wall by increasing ventilation and, therefore, O_2 uptake at the hindgut during hypoxia.

56.5

TEMPERATURE AND BLOOD FLOW IN PIGEON BRAIN DURING HEAT AND HYPOXIA.

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

Birds regulate the temperature of the brain (T_b) and of the body core (T_c) 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 T_b and CBF, pigeons (*Columba livia*, mass 0.35 kg) were kept thermoneutral or were heated, and were given normoxic air (PO_2 138 Torr) or N_2-diluted air (PO_2 85 Torr). We measured T_c and T_b with thermocouples in the colon and hypothalamus and estimated relative CBF from H_2-washout rates, monitored with a Pt electrode in the hypothalamus. During heat stress alone, T_c and T_b increased, but so did the difference (ΔT) between them. During hypoxia ΔT was always decreased, from about 2.5°C to about 1.5°C. In thermoneutral hypoxic birds this was due to a fall in T_c while T_b remained unchanged. In heat-stressed hypoxic birds it was due to a rise in T_b toward an unchanging T_c. 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 O_2 supply awaits further study. (Supported by NSF grant BSR8806604.)

56.6

SULFIDE OXIDATION IN THE MITOCHONDRIA OF *ARENICOLA MARINA*.

Susanne Völkel 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 (Völkel, 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 (Völkel, S., Grieshaber, M.K. (1994) Mar. Biol. 118, 137). Sulfide oxidation was coupled 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 6fold less sensitive to cyanide than respiration with succinate as the only substrate. The 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. (Supported by the Bundesminister für Forschung und Technologie, Germany, under the project "DYSMON" 03F0123B)

TEMPERATURE ADAPTATION AND ENERGETICS

57.1

TEACHING PRINCIPLES OF ANIMAL BEHAVIOR AND PHYSIOLOGY WITH A TEMPERATURE GRADIENT.

C.S. O'Connor* and L.I. Crawshaw. Biology Dep't, Portland State Univ., Portland OR 97207

All animals seek to maximize survival and optimize function by maintaining a favorable body temperature. To achieve this, all mobile animals rely upon behavioral thermoregulation which can be observed, manipulated, and quantified 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. Set point for body temperature of mammals may be lowered (i.e. by injection of the drug, ethanol) or raised (i.e. by injection of pyrogen); such alterations 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.

57.2

NATURAL POTENTIAL BODY TEMPERATURES OF NON-ADULT *DROSOPHILA MELANOGASTER* IN RELATION TO HEAT-SHOCK PROTEIN EXPRESSION .

Martin Feder, Nathaniel Blair*, and Hunter Figueras*, Univ. of Chicago, Chicago, IL 60637.

Although *Drosophila melanogaster* is a major experimental model for the heat-shock response in eukaryotes, little is known of the natural thermal 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, presumably the natural habitat of the eggs, embryos, larvae, and pupae of *Drosophila melanogaster*. We used thermocouples to monitor temperatures of rotting peaches, nectarines, apricots, and tomatoes placed at selected natural and semi-natural sites in Cook County, IL, in June and July 1994. Rotting fruits on grass or soil substrates routinely exceeded 35° C after 75-90 minutes of insolation; temperatures > 40° C were not uncommon and some tomatoes exceeded 50° C. Except in tomatoes, thermal heterogeneity within intact fruits was small, typically < 4° C. Variation in equilibrium temperatures of rotting fruit and the kinetics of heat exchange are due in part to differences in insolation, fruit color, evaporative water loss, insolation of the substrate prior to fruit placement, and fruit mass. On cloudy days or in sustained deep shade thermal heterogeneity is modest, and ovipositing females cannot use fruit temperature as a cue to avoid fruits that may present lethal heat stresses on subsequent days. These findings, in conjunction with published data on heat-shock in *Drosophila melanogaster*, suggest that non-adults of this species may routinely experience temperatures sufficient to induce expression of heat-shock proteins and to which heat-shock proteins ought to confer tolerance. Supported by NSF IBN-9408216.

57.3

TEMPERATURES THAT ELICIT PEAK METABOLIC RATES: STATISTICAL DISTRIBUTIONS AND IMPLICATIONS. Richard W. Hill, Patrick E. Lederle*, and Donald L. Beaver*. Michigan State University, East Lansing, MI 48824

Investigators typically employ a single ambient temperature to elicit cold-induced peak metabolic rates of recently captured mammals and birds. The temperature used in a particular study presumably was selected through exploratory tests, but data from such tests are usually not reported. Lack of published data from exploratory tests precludes evaluation, and use of a single ambient temperature could fail to recognize important individual variation. Increased knowledge of the statistical distributions of temperatures that evoke peak metabolic rates is needed to place studies of peak rates on a firmer foundation. As part of a larger project, 146 deer mice (*Peromyscus maniculatus*) and 158 black-capped chickadees (*Parus atricapillus*) caught in winter were tested at multiple ambient temperatures (5 C apart) soon after capture to determine the particular temperature required to elicit peak metabolic rate in each individual. The heliox method was used. In both species, the distribution of temperatures that elicited peak metabolic rates in various individuals was quasinormal. Most chickadees exhibited their peaks at 0, -5, -10, or -15 C; the span of temperatures for mice was similarly wide but lower: -5, -10, -15, or -20 C. Members of both species were sufficiently variable that use of any single ambient temperature could cause substantial misestimation of peak metabolic rates of particular individuals. The population mean, however, could be estimated well enough for many purposes using only one or two test temperatures. The mean metabolic rate of all chickadees at -5 C, for example, was only 5% lower than the mean of their respective true peaks. Supported by U.S. Navy Submarine Communication Project Office, contracts N00039-84-C-0070 and N00039-88-C-0065.

57.4

GLOBAL WARMING AND ACID RAIN: THE EFFECTS OF ELEVATED WATER TEMPERATURE, LOW PH, AND RATION LEVEL ON APPETITE, GROWTH, AND METABOLISM IN RAINBOW TROUT. Jacqueline J. Dockray, Teresa D. Banka*, Scott D. Reid, D. Gord McDonald and Chris M. Wood. McMaster University, Hamilton, Ontario, Canada, L8S 4K1

The combined effects of increased global temperatures and acid rain pose a potential threat to Canada's freshwater fisheries. To assess these conditions on juvenile rainbow trout (*Oncorhynchus mykiss*), a 3 month long experiment was conducted in soft water (Na^+= 57.7±2.3, Ca^{2+}= 97.5±2.2 µequiv/l) over the period June - September 1993. Trout were exposed to combinations of the following: ambient water temperature (range 13-24°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. Trout exposed to low pH had greater appetites 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 rates 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)

57.5

EFFECTS OF TEMPERATURE ON METABOLIC AND ACID-BASE RESPONSES IN TROUT. James D. Kieffer, Suzanne Currie and Bruce L. Tufts. Biology, Queen's University, Kingston, Canada. K7L 3N6.

In vivo experiments were conducted to determine how the physiological response to exhaustive exercise in rainbow trout is affected by temperature. The white muscle acid-base status (e.g. pH) and metabolite (e.g. lactate, phosphocreatine, (PCr), ATP and glycogen) content, and the acid-base status and lactate concentrations in the blood, were measured at rest and during recovery from burst exercise in trout acclimated to either 5 or 18°C. Trout acclimated to 18°C had higher resting levels of white muscle PCr and also utilized more ATP and glycogen stores during burst activity compared with trout acclimated to the colder temperature. Recovery of muscle PCr and glycogen levels was independent of temperature, but ATP recovered more quickly at 18°C. Exhaustive exercise resulted in a similar lactacidosis in the muscle of trout acclimated to either temperature. In contrast, temperature had a marked influence on the lactacidosis in the blood. Blood lactate and metabolic proton concentrations following exercise were about twofold greater in fish acclimated to 18°C than in fish acclimated to 5°C. Despite the more severe acidosis and the greater lactate accumulation in the plasma of fish acclimated to warmer temperatures, the time required for recovery of these variables was very similar to that at 5°C. Taken together, these results suggest that acclimation temperature does not significantly affect anaerobic capacity in rainbow trout, but may account for much of the documented variability in the dynamics of the lactacidosis in blood following exhaustive exercise in fish.

57.6

MECHANISMS OF REGULATORY VARIATION IN THE LACTATE DEHYDROGENASE-B GENE WITHIN AND BETWEEN POPULATIONS OF A TELEOST FISH: FUNDULUS HETEROCLITUS. Patricia M. Schulte and Dennis A. Powers. Hopkins Marine Station, Pacific Grove, CA 93950

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 F. heteroclitus which are exposed to substantially different environmental temperatures. These differences, which persist on acclimation to a common temperature, have been shown 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 DNase 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-9022648.

57.7

TROUT PLASMA MEMBRANE CHOLESTEROL CONTENT IS CORRELATED WITH ACCLIMATION TEMPERATURE. John C. Robertson and Jeffrey R. Hazel. Arizona State Univ., Tempe, AZ 85287

Compensatory changes in lipid composition allow ectotherms to maintain functional cellular membranes with ambient temperature change. Cholesterol is a major plasma membrane component of many eukaryotic cells and is known to influence the physical properties of bilayer membranes. Thus, cholesterol would appear well-suited to play a role in thermally-evoked plasma membrane lipid restructuring. To explore this possibility, we measured the relative cholesterol content of plasma membranes isolated from several tissues of warm (20° C) and cold (5° C) acclimated rainbow trout (Oncorhynchus mykiss). Mean cholesterol:phospholipid molar ratios were significantly higher in membranes from warm versus cold acclimated fish in liver (0.26 vs. 0.18; p =.007), kidney (0.49 vs. 0.40; p =.018) and gill (0.66 vs. 0.60; p =.0498); in erythrocytes, the difference was not significant (0.28 vs. 0.25; p =.253). These results suggest cholesterol modulation may be involved in the thermal homeoviscous response of poikilotherm plasma membranes. Studies in model membrane systems indicate cholesterol stabilizes fluid phase membranes; increasing the cholesterol content of plasma membranes may thus counterbalance the membrane-perturbing effects of higher temperature. Our data also reveal apparent tissue differences in both plasma membrane cholesterol levels in trout and the extent of temperature-associated shift in cholesterol content; these variations may reflect unique tissue functions. Supported by NSF IBN 9205234.

57.8

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

Short-horned sculpins, *Myoxocephalus scorpius*, were acclimated to 5 and 15°C to evaluate the impact of acclimation temperature upon the thermal sensitivity of mitochondrial regulatory properties and maximal 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. At their respective acclimation temperatures, rates of substrate oxidation by the isolated mitochondria were similar. Cold acclimation of sculpins did not alter the ADP affinity of mitochondria at low temperatures but markedly increased the Km_{app} values at 12.5 and 20°C. At the acclimation temperatures, mitochondrial ADP Km_{app} values did not differ. When compared with mitochondria isolated from red muscle of fish living at a wide range of temperatures, maximal rates of substrate oxidation and ADP affinities of mitochondria from cold-acclimated sculpin suggest compensatory thermal adjustments of mitochondrial properties have occurred during evolutionary specialization to different thermal habitats. Supported by funds from NERC to IAJ and NSERC to HG.

57.9

METABOLIC RESPONSES OF THE SOUTH AMERICAN TELEOST *PROCHILODUS SCROFA* TO HYPOXIC CONDITIONS UNDER DIFFERENT TEMPERATURES. Wilma R. Barrionuevo*; Marisa N. Fernandes* and Francisco T. Rantin* Rua Sao Sebastiao, 1455. Sao Carlos, SP, Brazil 13561-170. E-mail: pwrb@iris.ufscar.br - Universidade Federal de Sao Carlos.

Respiratory variables were measured for the Prochilodontid fish, *Prochilodus scrofa*, acclimated to 15°, 20°, 25°, 30° and 35°C, and exposed to graded hypoxia. Oxygen consumption ($\dot{V}O_2$) increased with increasing of temperature but at each temperature the $\dot{V}O_2$ was maintened constant over a wide range of PO_2s. The critical oxygen tensions (PcO_2) were 28, 22, 22, 24 and 45 mmHg for fish acclimated to 15°, 20°, 25°, 30° and 35°C, respectively. Gill ventilation ($\dot{V}_G$) increased in relationship with the temperature and hypoxia as a result of an accentuated increase in breath frequency (f_R) and breath volume ($V_{S,R}$). $\dot{V}_G$ and $V_{S,R}$ decreased in very high hypoxic levels at 35°C. Oxygen extration was kept constant in normoxia and moderate hypoxia (PO_2~70) regardless of the temperature. *P. scrofa* showed high tolerance to hypoxia under different temperatures although this effective performance could become limited by the capacity of ventilatory mechanisms to alleviate hypoxic stress under higher temperature levels.

57.10

CONTROL OF FEEDING: A STRATEGY OF OVEREATING. Robert N. Stiles. Univ. Tenn., Memphis, TN 38163

Control of feeding is generally considered homeostatic, with models named for the regulated variable, *e.g.*, glucostatic, lipostatic, etc. Energy balance may occur independently or as a result of regulation of body fat. Obesity may result from an elevated set point in fat regulation, or from accumulation of small positive errors in energy balance. Adult humans (especially males) tend to maintain energy balance with a yearly precision of about 0.2%. Weigle states (FASEB J. 8, 1994) that this precision indicates " . . . the operation of a system that continually matches intake and expenditure." However, it is unclear how intake and expenditure are continually monitored. Also, in humans, this match may not occur on a daily, or even a weekly, basis. Certainly fat regulation could maintain energy balance over long periods without continual monitoring of intake and expenditure. Another possible mechanism (proposed here) is that control of feeding is similar to control of fueling of a vehicle. Vehicle fueling is driven by the imperative "don't let the fuel tank go empty." This fueling strategy is one of overfueling rather than compensation (regulation). Given this strategy and a fixed storage capacity, energy balance will result. Animals of different species continually go through periods of overeating (feeding periods) followed by periods of undereating (non-feeding). Given this, body fat may represent a store (an auxiliary store) of calories for future periods of non-feeding (Le Magnen and co-workers). While fat may appear to be regulated, its steady-state value may result as a balance between factors controlling reesterification and lipolysis, factors such as insulin levels and fat cell size, respectively. Obesity may reflect this balance, rather than occur as a result of overeating.

57.11

OXIDATIVE FUEL METABOLISM OF THE VIRGINIA OPOSSUM IN PROLONGED EXERCISE AND FASTING. Jean-Michel Weber, *Cassandra Grant and *Timothy O'Connor. Biology, University of Ottawa, Ontario, Canada K1N 6N5

Rates of oxygen consumption (VO_2), CO_2 production (VCO_2), and urea nitrogen excretion (N) 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 respirometer for 6 days (fed 3 days/fasted 3 days), while urine was collected daily. During exercise, protein oxidation only played a minor role (<6% VO_2). At the onset of work, more than 90% of the energy came from the oxidation of small carbohydrate reserves. After 20 min, carbohydrate oxidation started to decline progressively while lipid oxidation increased concurrently until the importance of these 2 fuels became equal towards the end of exercise. In resting animals, protein oxidation accounted for 18% of VO_2, and this value was not changed by fasting. In contrast, a 3-day fast caused a steady increase in lipid oxidation (52 to 77% VO_2) and a major decline in carbohydrate oxidation (30 to 6% VO_2) . 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.

57.12

OXYGEN CONSUMPTION, BODY TEMPERATURE AND VENTILATION IN BLACK-CAPPED CHICKADEES DURING ACUTE COLD STRESS. Daniel T. Clemens. Department of Biology , Williams College, Williamstown, MA 60637.

Black-capped chickadees were exposed to short bouts of severe cold stress, using a 79.5% helium/20.5% oxygen (HeOx) gas mixture in an open-circuit respirometry system. Peak rates of oxygen consumption (V_{O2}max) in HeOx were determined by lowering ambient temperature (T_a) in discrete steps from 5°C, so that steady state V_{O2} was attained at each T_a in HeOx followed by recovery in air. Body temperature (T_b) was measured continuously by intraperitoneal thermocouple, and ventilatory frequency (f_B) was measured by body plethysmography. Each bird in HeOx was measured down to a T_a at which V_{O2}max and T_b began to decline rapidly. Standard V_{O2} and f_B in air were measured in late summer; cold stress measurements in HeOx were done in early fall and mid winter.

Standard V_{O2} (at 28°C) and thermal conductance in air were close to allometrically predicted values. Thermal conductance decreased with decreasing T_a between 5 and -10°C, and averaged19% lower in winter than in early fall. V_{O2}max in HeOx averaged 4.8 times the standard V_{O2}, and did not differ significantly between fall and winter birds. However, winter birds maintained stable T_b's and reached V_{O2}max at lower T_a's than did fall birds. Mean T_b at V_{O2}max was higher in winter (37.6°C) than in fall (35.9°C), and V_{O2}max was positively correlated with T_b, with a temperature quotient (Q_{10}) of 1.45. Mean f_B in air increased linearly with decreasing T_a, from 52 breath/min at 28° to 89 breath/min at -10°C. In HeOx, f_B was positively correlated with peak V_{O2} and averaged 166 breath/min at V_{O2}max.

These findings suggest that reduction of thermal conductance, rather than increase in metabolic scope, is important for seasonal cold adjustment in this species. Furthermore, the low Q_{10} of V_{O2}max could be important in facilitating arousal from nocturnal hypothermia at very low Ta's.

57.13

DIVING ENERGETICS AND OXYGEN ECONOMY IN FORAGING DUCKS. Richard Stephenson, Dept. of Zoology, University of Toronto, Ontario, Canada M5S 1A1.

A closed-circuit respirometry system was used to study biomechanical and metabolic power of unrestrained diving lesser scaup, Aythya affinis. Mechanical power output and aerobic power input (oxygen consumption) were 3.69±0.24 and 29.3±2.5 $W.kg^{-1}$, 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 net acceleration contributed 11% during descent and 0% (assumed) during the feeding phase. Buoyancy caused by air in the respiratory system and plumage layer fell from 6.2±0.4 $N.kg^{-1}$ to 4.2±0.3 $N.kg^{-1}$ during the dive due to air loss from the plumage and hydrostatic compression of the remaining air. Hence, these gas compartments strongly 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.

57.14

LOW RQ VALUES IN HUMMINGBIRDS UNDER FASTING CONDITIONS. J.E.P.W. Bicudo and J.G. Chaui-Berlinck*. Department of Physiology, University of São Paulo, Brazil.

Hummingbirds have one of the highest mass specific oxygen uptakes amongst vertebrates. Their feeding is based mainly on nectar, resulting in a great ingestion of carbohidrates. Soon after the last feeding their respiratory quotient (RQ) reaches values above unity, indicating conversion of carbohidrates into fat. Measurements of RQ of hummingbirds fasting for about 2 or 3 hours show values around 0.67, presumably indicating that they are utilizing stored fat. Our results from hummingbirds submitted to even longer fasting periods show RQ's dropping even further, reaching values as low as 0.3. Their oxygen uptake during these periods varied between 3-14 ml $O_2\ g^{-1}\ h^{-1}$, and their body temperature was about 37°C. Such low RQ values have not been described for both avian and mammalian species. For the appearance of such low RQ's we propose the formation of ketone bodies. However, ketone bodies should not be fully utilized by the organism. The end-result of this could be a profound ketoacidosis. Ketone bodies could be important for those tissues that utilize glucose as their primary energy substrate, such as the central nervous system. At the same time, they might prevent the catabolism of skeletal muscle proteins which are indispensable for nectar feeding in hummingbirds. Supported by FAPESP and CNPq, Brazil.

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 (T_{ab}) than temperate queens when foraging and nest-hunting soon after they emerge from hibernation in the spring. Elevated T_{ab} accelerates ovary development and colony-founding thereby allowing arctic bumblebees to establish colonies during the short arctic summer. The energy required to maintain high T_{ab} is in part determined by insulation. We determined the insulating capacity of bumblebee pile. In general, arctic (from Alaska) queen bumblebees had lower abdominal cooling constants 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 ULTRASTRUCTURE IN PECTORALIS MUSCLE OF PIGEON. Q. Mathieu-Costello, P.J. Agey, K. Rousey and M.H. Bernstein. Dept. of Medicine, UCSD, La Jolla CA 92093-0623, and Dept. of Biology, New-Mexico State Univ., Las Cruces, NM 88003-0032

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 69 days. The muscles were perfusion-fixed *in situ*, processed for electron microscopy and analyzed by morphometry. There was no significant difference in body weight over the cold exposure period. The volume fraction of lipid droplets in aerobic fibers was 4-fold greater in cold-exposed pigeons (group mean, 7.8 ± 0.9%), compared to normothermic sedentary (2.2 ± 0.7%) or wild-caught pigeons (1.7 ± 0.2%), 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 intrafiber lipid deposition were all closely correlated in cold-exposed pigeons. The results suggest that the closely matched aerobic capacity and vascularization of the highly oxidative fibers in pectoralis muscle of pigeon were sufficient to cover the increased energetic demand with shivering. Chronic exposure to cold did not alter capillary-fiber structure in the muscles. Supported by NIH PO1HL17331 and NSF DEB-9270148.

57.17

ELECTROCARDIOGRAPHIC EVIDENCE FOR CARDIAC HYPOXIA DURING EXTREME HYPOTHERMIA IN NEONATAL *Peromyscus leucopus*. Bradley A. White* and Richard W. Hill. Michigan State University, East Lansing, MI 48824

White-footed mice (*Peromyscus*) under 10 d of age tolerate near-freezing body temperatures (2-3 C) for several hours with impunity. Breathing terminates within the first hour of hypothermia, resuming 15-20 min after rewarming has begun. During rewarming, electrocardiograms (EKGs) often display a dramatic acceleration of the heart rate soon after the first one or two breaths. Since several mechanisms could be responsible for this phenomenon (cardiac reoxygenation, thermal effects, stimulation by breathing mechanics), an experiment was designed to test whether renewed O_2 availability upon breathing is critical for heart rate acceleration. Mice were exposed to an ambient temperature of 2-3 C for 3 h and then returned to 20 C. Each mouse was enclosed in a glass chamber which permitted the regulation of gas exposure. During rewarming, the mouse was exposed to either air or N_2 immediately prior to and throughout the first 2 min of breathing. Each mouse was treated more than once and used as its own control. Heart rates from EKGs were quantified as the number of ventricular complexes per min during rewarming before and after breathing resumed. Postbreath heart rate acceleration occurred consistently during air but rarely during N_2 exposure (mean ratio of post-:pre-first-breath rates = 1.7 for air, 0.6 for N_2). Heart rates after the first breath were higher overall in air than N_2 (mean ratio = 6.2). Sinoatrial pacing and rhythmicity tended to stabilize after first breaths in air, but not in N_2. Thus, O_2 availability is believed to be the primary cause of heart rate acceleration seen after breathing resumes during rewarming. Hypoxia occurs during bouts of near-freezing hypothermia, making the normality of subsequent development all the more interesting.

57.18

EVALUATION OF THE SUSCEPTIBILITY TO FROSTBITE USING INFRARED IMAGING. Michel B. Ducharme, Sydney D. Livingstone and Allan A. Keefe. Defence & Civil Institute of Environmental Medicine, North York, Ont. Canada, M3M 3B9.

The Frostbite Point Test Method or Yoshimura Test is the best known and accepted test to evaluate the resistance to frostbite. The test consists of a 30-min immersion of the middle finger of the dominant hand in a bath maintained at 0°C during which the vascular response of the finger to cold is quantified from the measurement of skin temperature changes at the distal phalanx. The objective of the present study was to develop an equivalent test in air which would be less stressful on the test subject. For this new test, the subjects were seated in an environment of 25.0±0.1°C with 2 x 250 W infrared lamps aimed at their back at a distance of 0.75m. Their hands were placed in a box in which the air was maintained at 5.0±0.2°C. Skin temperature at the tips of the ten fingers and at the back of the hands were recorded every 10 min using an infrared imaging camera. 31 male and female subjects representing a good range of frostbite susceptibility (from 3 to 9 on the Frostbite Resistance Index) did both the Yoshimura and the air tests. A significant ($p<0.001$, $r=0.6$) linear relationship was found between the Frostbite Resistance Index calculated from the Yoshimura Test, and the average temperature difference between the back of the hands and the finger temperatures that were observed during the 30-min cold exposure. A greater temperature difference between the hands and fingers during the test appears to indicate a greater susceptibility to frostbite. We conclude that the new test may be useful in predicting susceptibility to frostbite in air.

HEART AND CIRCULATION

58.1

EFFECTS OF RACING ON HEMATOLOGY AND VISCOELASTIC PROPERTIES OF HORSE BLOOD. Michela Baca, Stephen Wood, and M. Roger Fedde. Cardiopulmonary Physiology Program, The Lovelace Program, The Lovelace Institutes, Albuquerque, NM, 87108.

The splenic contraction of horses during racing increases the hematocrit, e.g., 40 to 65%, producing a dramatic rise in blood viscosity (Fedde & Wood 1992, FASEB, 6:A1529). No previous data were available on the effect racing has on blood elasticity. We tested the hypothesis that known changes (via an acute phase response) in horse blood during strenuous exercise, e.g., increased hct and fibrinogen would increase aggregation and decrease deformability of RBCs, key parameters in the elastic properties of blood. Basic hematology (Coulter Counter) and viscoelasticity (oscillatory flow viscometer; Vilastic 3) were measured in blood from 12 horses before and after racing. All horses had leukocytosis and erythrocytosis, independent of race distance (0.32 to 1.7km). Elastic Yield Stress is the point at which blood is transformed to a superfluid state (Thurston, 1989, Biorheology, 26:199) and depends on RBC aggregation and deformability. Post-race horses had an EYS of 1.1 dynes/cm^2 compared with 0.3 pre-race, due largely to the increase in hct from 39 to 64%. However, at constant hct the blood of post race horses still had a significantly elevated EYS, possibly due to increased fibrinogen. This elevation would be relevant and deleterious in circulation characterized by pulsatile flow, e.g., the coronary circulation and exercising muscle. *Research supported by NIH Grant HL 40537.*

58.2

FUROSEMIDE DOES NOT AFFECT THE RATE OF RISE OR UNSTEADINESS OF PULMONARY VASCULAR PRESSURES IN THOROUGHBREDS (TB) PERFORMING RAPID ACCELERATION SUPRAMAXIMAL (SM) EXERCISE (EX). Murli Manohar, Univ. Illinois Coll. Vet. Med., Urbana, IL 61801

In competitive racing, TB rapidly accelerate to their top speed and perform SM work. Thus, our objective was to examine pulmonary vascular pressures of TB with rapid acceleration to supramaximal EX which could not be sustained for >90s. Right atrial and pulmonary vascular pressures were studied in 10 healthy, sound, fit TB during SM-EX performed on separate days without and with furosemide (250mg IV, 4 hrs pre-EX). A rapid acceleration EX protocol was used, in which speed was increased from 8 to 15 m/s in 8s. All horses performed 90s of SM-EX @ 15m/s + 10% uphill incline. Rapid acceleration of TB was attended by an equally rapid escalation in right atrial and pulmonary arterial, capillary and venous pressures such that all pressures had attained their zenith at the very onset of SM-EX. Peak values of various pressures were maintained only for the first 30 - 45s of SM-EX. Thereafter, a declining trend emerged and all pressures decreased significantly as EX duration increased. Furosemide significantly lowered the pulmonary vascular pressures at rest and during EX, but it did not affect the rate of rise or the unsteadiness of pulmonary vascular pressures with rapid acceleration to SM-EX. It is suggested that the rapid rate of rise of pulmonary capillary blood pressure during a sudden burst of supramaximal EX may be a crucial factor in precipitating stress failure of pulmonary capillaries and initiation of exercise-induced pulmonary hemorrhage very early in the course of a race.

58.3

HEMODYNAMICS OF RATS AND MICE. Robyn L. Phelps, David R. Jones, and Arnold T. Mosberg*. UBC, Vancouver, BC., V6T 1Z4, CANADA

Vertebrate hemodynamics can be described by the *Windkessel* and *Tubular* models. The *Windkessel* is a lumped parameter system which experiences no discrete wave reflection effects. The *Tubular* model describes incident and reflected wave interactions which augment pressure but reduce flow pulsations peripherally. Wave velocity (C), cardiac frequency (f_o), and arterial length (L) determine the timing and degree of wave propagation effects. Most mammals are described as *Tubular* however, small species may be mismatched with regard to $C/L/f_o$. Aortic arch 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 $C/L/f_o$ 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 murine cardiovascular system is characteristic of other mammalian species, it can be described as a functional *Windkessel* due to a $C/L/f_o$ 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 circoelectric 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. Milesi*, L. Biancardi*, °D. Pendergast, A. Veicsteinas
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 at steady states of cycle exercises of increasing loads up to exhaustion in 22 °C air (A) and 30 °C water (W) in 7 trained male swimmers (24±3 yrs). It resulted that: a) HR from resting values of 54±3 b/min in W and 64±4 b/min in A ($p<.05$) increased linearly with oxygen uptake ($\dot{V}O_2$) with similar slopes both in W and A. Maximal oxygen uptake ($\dot{V}O_{2max}$) was 3.7±.1 l/min and 3.8±.2 l/min in W and A, resp. ($p>.05$); b) at rest the power of low frequency component (LF, .05–.15 Hz), as % of the total, was 25.8±4.7% in W and 34.9±4.1% in A ($p>.05$). With exercise both in W and A LF% increased to 40±5% and was unchanged up to a $\dot{V}O_2$ of 2 l/min, then decreased linearly to 0; c) the % power of high frequency component (HF, .15–1.0 Hz) was at rest similar in W and A (23.5±4.7%). During exercises both in W and A, HF% decreased to 10±3% ($p<.05$) up to 70% $\dot{V}O_{2max}$, then it increased to 25±3%. The HF central frequency increased from .25 Hz at rest to .68 Hz at $\dot{V}O_{2max}$. The different blood distribution due to immersion did not affect the power spectrum of HRV at rest or during exercise. The changes observed during exercise are thus due to the readjustment in circulatory and respiratory systems and seem to depend mainly on metabolic demand.

58.6

VASOCONSTRICTION 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 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 anaesthetised elasmobranchs, *Heterodontus portjacksoni, Hemiscyllium ocellatum and Rhinobatos batillum*. In addition, contraction of the isolated pancreatico-mesenteric artery in an organ bath was measured in response to increasing doses of galanin (to 10^{-6}M). Galanin caused a significant rise in mean caudal arterial BP in *H. portjacksoni* ($P<0.001$,n=6) and *H. ocellatum* ($P<0.01$,n=7), but no change in *R. batillum*. Galanin (10^{-6}M) caused 30-40% of the maximum K^+ induced contraction in the isolated gut artery in all species. In *R. batillum*, 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

A POSSIBLE MECHANISM OF FLOW AUTOREGULATION IN GLOMERULI OF HAGFISH. J. A. Riegel, Zoology Dep't, Cambridge University, England, CB2 3EJ

In glomeruli of lightly-anaesthetised hagfishes capillary pressures average c. 2 cm water with a maximum value of c. 4 cm water. Average pressures measured in glomeruli isolated and perfused with Ringer were similar. In the latter case, however, it was possible to detect two kinds of vessels whose pressure responses varied with flow. The first kind, 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 matching the perfusion pressures. It is postulated that the responses of the two kinds of glomerular capillaries form part of a mechanism for autoregulation of flow through the hagfish glomerulus.

58.8

UPDATE ON THE COMPARATIVE PHYSIOLOGY OF PULMONARY INTRAVASCULAR MACROPHAGES. KE Longworth, DE McClure, A Nicolaysen, KA Jarvis, BL Smith and NC Staub. Univ of California, Davis, 95616 and San Francisco, 94143; The Ohio State Univ, Columbus, 43210; Univ of Oslo, Norway.

In response to intravenous Monastral 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, 1986; *FASEB J* 6:A1242, 1992). We have extended our studies to other species (shown in **bold**).

Order/species		Lung MB retention	Increase in PA pressure	Intravascular macrophages containing MB
Artiodactyla	**llama**	*Y*	*Y*	*Y*
sheep, reindeer, cattle, goat		*Y*	*Y*	*Y*
Perissodactyla	horse	*Y*	*Y*	*Y*
Carnivora	**dog**	N	N	N
	ferret	N	N	N
	cat	*Y*	N	*Y*
Primates	**monkey**	N	N	N

Only species with pulmonary intravascular macrophages show an increase in PA pressure after Monastral 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 Perissodactyla.[Support: Amer Heart Assoc (KEL) & HL25816 (Prog Proj)].

58.9

STATIC MUSCULAR CONTRACTION ELICITS A PRESSOR REFLEX IN THE CHICKEN. T.P. Adamson* 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 responses. We designed 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 13 chloralose anesthetized cockerels, we evoked static contractions in 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. Static contraction of the gastrocnemius increased MAP from 71±4 to 95±4 mmHg ($p<0.05$), and increased HR from 304±8 to 345±10 BPM ($p<0.05$). The same stimulus parameters after paralysis with vecuronium bromide (n=7) or after cutting the nerve distal to the stimulating electrode (n=1) evoked no change in MAP or HR. Further, stimulating the peripheral end of the cut the sciatic nerve generated equivalent tensions but no change in MAP or HR (n=5). We conclude that static contraction of the gastrocnemius muscle in the chicken elicits a pressor response, and that this response is a neurally mediated reflex arising from the contracting muscle.

58.10

ISCHEMIC 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 30 min show extensive damage manifest by loss of mechanical function and leakage of intracellular enzymes upon reperfusion. If all or part of the coronary circulation is occluded briefly (10 min) and flow restored prior to the prolonged occlusion, 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 cardioprotective mechanisms than the laboratory 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 LDH 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 R15HL46506.

58.11

THE STRUCTURE AND FUNCTION OF THE BULBUS ARTERIOSIS OF THE MARINE CLAM, *Mercenaria mercenaria (L.)*. Daniel W. Duhon, Bruce E. Felgenhauer* and Lewis E. Deaton. Department of Biology, University of Southwestern Louisiana. Lafayette, LA 70504.

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 was examined using microscopic and pharmacological techniques. Light microscopy using Mallory's triple stain did not reveal the presence of striated 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 extracted bulbus arterioses were separated using a C-18 Sep-Pak cartridge and bioassayed for cardioactive agents using isolated ventricles. 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^{-7} M) induced contraction of the bulbus arteriosis. Thus, we propose a possible role for the bulbus arteriosis in cardiovascular function.

58.12

MORPHODYNAMIC ANALYSIS OF THE HEART OF *C. hamatus*, A HEMOGLOBINLESS ANTARCTIC FISH. Bruno Tota, Raffaele Acierno and Claudio Agnisola*. UNICAL, Arcavacata di Rende (CS), Italy

Most icefishes, with their lack of hemoglobin (Hb), cardiac myoglobin (Mb) (Vayda et al. 1994), high proliferation of muscle mitochondria, and very large blood volume, provide a challenging example of extreme adaptation to the cold highly oxygenated waters of the Antarctic (Eastman 1994). While the high mitochondrial content has been viewed as a homeostatic response to improve in the contractile tissues of these animals the transport of respiratory gases, ions and metabolites(Londraville and Sidell 1990), the large blood volume likely buffers the absence of Hb. As a consequence, the heart of these teleosts is under the synergistic actions of two powerful remodelling stimuli, one (mitochondrial growth) operating at subcellular level, and the other (volume overload) operating at the whole organ level. This results in a non-hypertrophic cardiomegaly characterized by myocyte enlargement due to mitochondrial overload and very low myofibril content. Our previous (Tota et al. 1991) and more recent mechanical studies, including image analysis (Tota et al. 1994), describe the geometric morphodynamic characteristics of the heart of the icefish *Chionodraco hamatus* in comparison with other red-blooded teleosts including the Antarctic *Trematomus bernacchii*. The results indicate that, in contrast with *T. bernacchii*, the icefish heart displaces remarkably high volumes of blood at high flow rates and low pressure, but is unable to adapt to increasing pressure load conditions, as can be expected on the bases of its low myofibrillar density. These data emphasize the adaptive cost in terms of myocardial performance in a cold milieu and in absence of respiratory pigments (Hb and Mb).

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58.13

CARDIOVASCULAR RESPONSES TO EXERCISE IN YELLOWFIN TUNA. K.E. Korsmeyer, N.C. Lai, H. Dewar, T. Knower, R.E. Shadwick, and J.B. Graham.. Scripps Institution of Oceanography, UCSD, La Jolla, CA, 92093-0204.

Yellowfin tuna (*Thunnus albacares*) were instrumented with electrocardiogram electrodes, ventral (VA) and dorsal aortic (DA) cannulae, and VA blood flow probes. Venous and arterial blood P_{O2}, O_2 content, pH, and lactate concentrations were determined. The tuna swam in a large water tunnel while swimming velocity was increased step-wise from 1.0 to 2.2 body lengths (BL)/s (48 to 105 cm/s). Heart rate increased from 62 to 98 bpm, mean VA pressure from 81 to 102 mmHg, and DA pressure from 50 to 58 mmHg. Venous O_2 content was 9.5 mlO_2/dl at 1 BL/s and decreased by 20% at the highest velocity, leaving a substantial venous O_2 reserve. Venous P_{O2} was maintained at 40 mmHg due to a drop in pH from 7.80 to 7.72 and the resulting Bohr shift. Plasma lactate increased from 0.8 to 2.4 mM. Increases in both stroke volume and heart rate contributed to an elevation in cardiac output. The high blood O_2 content may be further utilized for recovery from O_2 debt following anaerobic burst swimming or under conditions of environmental hypoxia. A model of O_2 delivery to red (aerobic) muscle suggests that the maximum sustained swimming velocity of yellowfin may be less than 3.5 BL/s.
(Supported by NSF OCE-91-03739)

58.14

CATECHOLAMINE AND POTASSIUM ION CONCENTRATIONS IN SWIMMING YELLOWFIN TUNA. N.C. Lai, K.E. Korsmeyer, J.B. Graham, R. Shabetai, and M.G. Ziegler. Veterans Administration Medical Center and University of California, San Diego, La Jolla, CA, 92093.

Blood samples were collected from 10 chronically instrumented yellowfin tuna (*Thunnus albacares*) while swimming in a large water tunnel. Assays of plasma catecholamine levels were done using the catecholamine-O-methyl transferase (COMT) method. Following surgery and at a swimming speed of 0.9 BL/s, norepinephrine (NE), epinephrine (E), and potassium ion (K^+) concentrations were 0.458 ± 0.388 nM (mean ± SD), 0.781 ± 0.298 nM, and 3.98 ± 0.30 mM, respectively. These levels declined and then remained relatively unchanged when the swimming velocity was increased step-wise from 0.9 to 2 BL/s (48 to 105 cm/s) over a period of six hours [0.73 ± 0.258 nM (NE), 0.676 ± 0.406 nM (E), and 5.61 ± 0.39 mM (K^+)]. In vertebrates, an increase in plasma potassium ion concentration resulting from skeletal muscle activity is known to trigger catecholamine release. However, no significant increase of catecholamines was seen in these tuna, either because the induced swimming velocities are still within the aerobic limit or there exists a biochemical pathway to prevent cardiac lesions due to the cardiotoxicity of high catecholamine concentrations.
(Supported by VAMC Merit Grant and NSF OCE-91-03739)

58.15

EFFECTS OF ARACHIDONIC ACID AND INDOMETHACIN ON TROUT HEART PERFORMANCE AND CORONARY CIRCULATION *IN VITRO*. Claudio Agnisola*, Tariq Mustafa, Rita Venzi, Frank B. Jensen* and Bruno Tota. Stazione Zoologica "A. Dohrn", I-80121 Napoli, Italy and Odense Univ., DK-5230 Odense M, Denmark

Arachidonic acid (AA), a common constituent of cell membrane 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 eicosanoids in the control of heart function in fish is presented. The study was performed on isolated and perfused trout (*Oncorhynchus mykiss*) heart preparations. Two perfusion set up were used and the effects of exogenous AA and the cyclooxygenase inhibitor indomethacin (IM) were evaluated. In the first set up the heart was perfused with a non re-circulating saline and a separate coronary head pressure. Heart performance and coronary resistance were time independent and AA induced negative inotropic effects, abolished by IM, and a strong increase of coronary resistance, which was reduced but not abolished by IM. In the second set up a small volume (3% of body mass) of re-circulating saline was used, and the coronary artery was connected to the cannulated ventral aorta, i.e. the coronary pressure was directly related to the pressure generated by the heart. In control conditions the hearts perfused in this way displayed a time dependent increase in the coronary resistance. This increase can be due to factors released by the heart and accumulated in the re-circulating saline. Interestingly the time dependence of coronary resistance was abolished by both AA and IM perfusion, suggesting the involvement of eicosanoids. The different results obtained with the two set up can be related to the interdependence existing between heart and coronary function in the re-circulating system, which in part reproduces the one occurring *in vivo*.

58.16

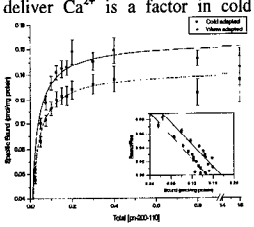
EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON WHOLE BODY AND TISSUE BLOOD AND EXTRACELLULAR FLUID VOLUMES IN THE RAINBOW TROUT. **D. W. Duff, P.G. Bushnell, D. J. Conklin, K.R. Olson. Biol. Dept. Ind. U. South Bend, IN 46634 & South Bend Ctr. for Med. Ed., Ind. U. Sch. of Med., U. Notre Dame, Notre Dame, IN 46556.**

Atrial natriuretic peptide (ANP) reduces plasma volume in mammals and circulating ANP increases during volume expansion. The effect of ANP on fluid compartments in teleost fish is unknown. In this study whole body and tissue blood volumes (^{51}Cr-red cell space) and extracellular fluid volumes (^{58}Co-EDTA space) were examined after 8 h infusion of saline (1.2 ml·h^{-1}·kg^{-1}) or ANP (300 ng·kg^{-1}·min^{-1}) in conscious rainbow trout (*Oncorhynchus mykiss*). ANP lowered whole body blood volume from 28.95±1.69 to 22.88±0.95 ml·kg^{-1} ($p\leq0.01$;n=22) and extracellular fluid volume from 242±17 to 169.6±5.8 ml·kg^{-1} ($p\leq0.001$;n=14). ^{51}Cr-red cell space was increased ($p\leq0.05$) by ANP in gills, brain, eye, pectoral fins, ventral muscle and bulbus and decreased in cecum. ANP decreased ($p\leq0.05$) ^{58}Co-EDTA space in gills, operculum, cecum, anterior and posterior intestine, swim bladder, anterior kidney, liver, gall bladder, pelvic fins, dorsal muscle and skull. These data suggest that ANP acts through renal or other volume-regulating epithelia to decrease central blood volume and produce a general extracellular dehydration, whereas local ANP-mediated vasodilatory responses may increase blood volume in certain tissues. **Supported by NIH grant R15 HL50088-01 (DD) and NSF grant IBN 9105247 (KO).**

58.17

CARDIAC DIHYDROPYRIDINE RECEPTOR (DHPR) DENSITY IS INCREASED IN THE COLD-ACCLIMATED TROUT *ONCORHYNCHUS MYKISS*. Brian Hamman* and Glen Tibbits. Cardiac Membrane Research Lab, School of Kinesiology, Simon Fraser University, Burnaby B.C., Canada V5S 1S6.

Trout can maintain high cardiac output both at warm (15-20°C) and cold (4-9°C) temperatures despite the negative inotropic effects of cold temperature. Adaptation to cold temperatures may be brought about by increasing: 1) the affinity of the contractile element (CE) for calcium (Ca^{2+}), 2) the number of CE, or 3) the ability of the myocyte to move Ca^{2+} across the sarcolemma (SL). To determine whether an increase in the ability of the trout heart to deliver Ca^{2+} is a factor in cold adaptation, saturation binding of the DHP [^{3}H] (+) PN200-110 to the DHPR of the SL Ca^{2+} channel was performed on ventricular homogenates of trout (*Oncorhynchus mykiss*) which had been acclimated for at least three weeks to either warm (17°C) or cold (7°C) temperature. Acclimation to cold temperatures resulted in an increased DHPR density or B_{max} (warm 0.137, cold 0.160 pmol/(mg protein), $p<0.05$) with no change in the affinity (see figure). The increase in DHPR density corresponds to 11.5 in warm- and 13.4 receptors/cm^2 in cold-acclimated trout. Since DHP receptors represent functional Ca^{2+} channels in cardiac muscle, an increase in B_{max} implies that cold acclimated trout are able to move more Ca^{2+} into the cell at the same temperature, improving contractility at lower temperatures. This study was funded by NSERC (Canada).



58.18

N^G-NITRO-L-ARGININE SUPPRESSES SOMATOSENSORY EVOKED POTENTIALS IN THE RAT. **Al C. Ngai*, Joseph R. Meno* and H. Richard Winn. University of Washington, Seattle WA 98104.**

Nitric Oxide (NO) synthase inhibitors such as N^G-Nitro-L-arginine (L-NA) have been shown to suppress cerebrovascular response to somatosensory stimulation, suggesting that NO may couple local blood flow and neuronal activity in the brain. Such a hypothesis remains controversial. On the other hand, there is evidence that NO is involved in neurotransmission. Thus, in addition to its vascular effects, inhibition of NO synthesis may affect neuron activity. We have investigated the effect of L-NA on somatosensory evoked potentials (SEPs) during stimulation of the sciatic nerve. Rats were anesthetized with urethane/chloralose, and a craniectomy was performed to expose the sensory cortex for SEP recording. The contralateral sciatic nerve was stimulated (0.2V, 5Hz, 0.5ms) for 20s before and during topical superfusion of 1 mM L-NA. Blood pressure remained stable during stimulation. After 30 min., amplitudes of both the P_1 and N_1 waves were reduced to 67±18% and 55±27% (n=4) of control, respectively. After 60 min., SEP amplitudes were further reduced to 42±12% and 25±14% of control. The NO synthase substrate L-arginine partially restored SEP amplitudes. L-NA had no effect on SEP latency. There was no change in SEPs in rats superfused with cerebrospinal fluid (>120 min., n=4). These results suggest that NOS inhibition markedly suppressed neuronal activity in cortex during somatosensory stimulation. (Supported by NS-21076).

58.19

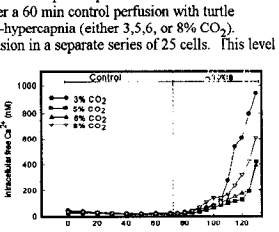
INOTROPIC ADAPTATIONS ON RATS SUBMITTED TO SWIMMING. Roseli Golfetti, Luiz E. Barreto Martins, Regina C. Spadari and Lourenço Gallo Jr.* Laboratório de Fisiologia do Exercício - FEF;Dept° Fisiologia - IB - UNICAMP, Campinas, SP, Brazil, 13081-970

Cardiac inotropism is stimulated by sympathetic division o neurovegetative system whose ends release mainly 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 adaptative responses of atrium to NE. The left atrium of Wistar adults rats were isolated and prepared for isometric contraction recording. Two groups of male Wistar rats were submitted to swimming in one acute session (50 min., ASW, n=7) and in three consecutive day sessions (5, 15, 30 min., ISW, n=7), in comparison with a control group (CO, n=8). The maximum isometric force, its maximum and minimum derivative, as well the pD_2 value were measured in response to increasing NE concentration, from 10^{-10} to 10^{-4} [mol]. The results did not show any statistical significant difference between the groups. Yet, the ASW group has shown an increased slope of dose-response curves to NE, suggesting a change in effector action of the tissue to NE induced by swimming Supported by FAEP and FAPESP (91/4754-9).

58.20

EFFECTS OF ANOXIA AND ACIDOSIS ON INTRACELLULAR FREE Ca^{2+} IN ISOLATED CARDIOMYOCYTES FROM TURTLES. **Jeremy S. Wasser and Norbert Heisler. Max-Planck Inst. for Experimental Medicine, D-3400 Göttingen, Germany.**

We examined the effects of anoxia and acidosis on cytosolic free calcium in isolated ventricular cardiomyocytes from painted turtles (*Chrysemys picta bellii*). These highly hypoxia tolerant animals have been the subjects of intensive study aimed at explaining their extraordinary ability to live without oxygen. We tested the hypotheses that: (1) isolated cardiomyocytes from painted turtles maintain intracellular Ca^{2+} homeostasis during anoxic exposure and prevent the rise in Ca^{2+} characteristic of mammalian heart cells; and (2) a moderate degree of acidosis improves the ability of turtle cardiomyocytes to maintain Ca^{2+} near control levels during anoxic stress ("pH paradox"). We isolated cardiomyocytes by enzymatic dissociation and loaded them with the acetoxymethylester of fura2. Cells were then allowed to settle onto glass cover slips in a perfusion chamber on the stage of an inverted epifluorescence microscope. After a 60 min control perfusion with turtle Ringer's, we subjected heart cells to 60 min of anoxic-hypercapnia (either 3,5,6, or 8% CO_2). Control Ca^{2+} averaged 27.6 nM after 60 min of perfusion in a separate series of 25 cells. This level was maintained for an additional 60 min (28.4 nM) and only rose to 46.7 nM after 2 hrs. This compares favorably with values obtained for mammalian cardiomyocytes (30 to 100 nM). The rise in cytosolic Ca^{2+} that we observed during anoxia with 3% CO_2 is comparable in degree and time course to that observed by other workers for quiescent, mammalian cardiomyocytes under similar conditions (Fig. 1). Increasing the perfusion CO_2 reduced the rise in Ca^{2+} with 5 and 6% having the best effect. We conclude that during anoxia with 3% CO_2, turtle heart cells did not display an extramammalian ability to prevent a rise in cell Ca^{2+}. With supplemental CO_2 at 5 or 6%, the anoxia associated increase in Ca^{2+} was blunted. This apparent protective effect was lost when CO_2 was further elevated to 8%. Supported by the Max-Planck Gesellschaft.



59.3

OSMOREGULATION BY DRINKING IN RATS.

T. Morimoto, E. Sugimoto* and H. Nose. Dept. of Physiol., Kyoto Prefectural University of Medicine, Kamigyoku, Kyoto 602, Japan

A method to measure circulating blood volume (BV) and plasma Na^+ concentration $[Na^+]$ continuously in rats was used to evaluate the rehydration 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 flow-through sodium sensitive glass electrode. Thermally dehydrated rats were provided with tap water and 1.8% NaCl and cumulative amounts of tap water and 1.8% NaCl intake were recorded for 4 h together with BV and $[Na^+]$. When both tap water and 1.8% NaCl solution were provided, the rat consumed mainly tap water in the first 45 min, and $[Na^+]$ recovered to the predehydration level within 60 min. Thereafter, the rat 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 decrease their blood osmolality. Thereafter, blood volume is expanded with consumption of almost isotonic NaCl solution. In other words, osmoregulation by drinking behavior precedes the volume regulation and sodium is required for complete recovery of blood volume.

59.4

ARGININE VASOPRESSIN (AVP) IS INVOLVED IN REGULATION OF EXPRESSION OF RAT KIDNEY UREA TRANSPORTER (rUT2) mRNA.

Craig P.Smith, Wen-Sen Lee[1], Jeff M.Sands[2], Sonia Martial[2], & Matthias A.Hediger[1]. [1]Dpt. of Med., Renal Div., Brigham and Women's Hospital & Harvard Med. Sch., Boston, MA; [2]Dpt. of Med., Renal Div., Emory University, Atlanta, GA.

In the mammalian kidney, regulation of urea transport is central to urinary concentration. Regulation of urea excretion and accumulation in the renal medulla depends on the functional state of specialized phloretin-sensitive urea transporters. To study these transporters and their regulation of expression we isolated cDNAs from rabbit (*Nature*, **365**:844-847, 1993) and rat (Smith et al., unpublished data) encoding functional urea transporters. Northern analysis of rat kidney mRNA revealed two transcripts of size 2.9 kb and 4.0 kb which had spatially distinct distributions. *In situ* hybridization of rat kidney sections and Northern analysis of rat kidney total RNA showed that manipulating the protein content of the diet or the hydration state of rats evoked defined, differential changes in the levels of the two transcripts. The 2.9 kb transcript was primarily responsive to changes in the hydration state, whereas the 4.0 kb transcript was primarily responsive to changes in the protein content of the diet. *In situ* hybridization on kidney sections from Brattleboro rats, which lack endogenous AVP, showed the same pattern of rUT2 mRNA expression as Long Evans controls. Water deprivation (24hr) had no effect on the pattern of rUT2 mRNA in Brattleboro rats. Kidneys from Brattleboro rats implanted subcutaneous with slow release pellets delivering 1 U AVP/day for 7 days showed a large increase in rUT2 mRNA. The pattern of rUT2 mRNA after AVP treatment was as observed in kidneys from water restricted normal rats. We conclude that AVP modulates rUT2 mRNA levels and that this action may in part be responsible for long-term regulation of rUT2 during water restriction.

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 hypotonicity-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'-diisothiocyanotostilbene-2,2-disulphonic 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 ATP[γS] and Mg^{2+} 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.

59.6

EFFECT OF CALCIUM CHANNEL ANTAGONISTS ON AMINO ACID RELEASE FROM BIVALVE VENTRICLES IN HYPOOSMOTIC MEDIA.

Lewis E. Deaton. University of Southwestern Louisiana, Lafayette, LA 70504

When exposed to hypoosmotic seawater (SW), the cardiomyocytes of marine bivalves release amino acids (AA) to regulate volume. In many cells, the initiation of solute release in response to hypoosmotic stress seems to be coupled to an influx of Ca^{++} ions. In bivalve cardiac muscle large Ca^{++} influxes occur with each contraction, and therefore initiation of volume regulation cannot involve a Ca^{++} influx. Isolated ventricles from the mussel *Geukensia demissa* were incubated in isosmotic (1000 mOsm) or hypoosmotic (500 mOsm) SW for 2 hr and the release of amino acids measured. The AA release in 1000 and 500 mOsm SW was 14 and 130 μmol/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, Co^{++}, La^{+++}, or Gd^{+++}. These results suggest that an influx of Ca^{++} ions is not involved in the control of AA release from bivalve myocardial cells in hypoosmotic SW.

59.7

REGULATORY VOLUME DECREASE (RVD) BY *NECTURUS MACULOSUS* RED BLOOD CELLS (RBC).

D. Light, L. Bergeron* and A. Stever*. Dept. of Biology, Ripon College, Ripon, WI 54971.

The lowest osmolality (mOsm/kg H_2O) RBC could be exposed to without hemolysis occurring was species dependent: *Necturus maculosus* (0.011), *Rana pipiens* (0.014), *Bufo marinus* (0.028), *Gallus gallus* (0.096), *Equus caballus* (0.140), *Homo sapiens* (0.150), *Ovis aries* (0.160). Exposure of *Necturus* RBC to hypotonic (0.5X) Ringer's produced a transient increase in volume followed by RVD. Incubating these cells in 0.5X high K^+/low Na^+ Ringer's inhibited RVD, suggesting that K^+ efflux was required. RVD also was inhibited by quinine and quinidine (1 mM). Gramicidin (1 μM) induced RVD when added to cells incubated in 0.5X Na^+-free Ringer's containing quinine, indicating quinine inhibited RVD by blocking a K^+ channel, and that Cl^- permeability was high. In contrast, gramicidin did not alter cell volume when added to cells bathed in 1X Na^+-free Ringer's, suggesting that basal Cl^- permeability was low. The protein kinase inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7, 10 μM) attenuated RVD and this effect was reversed by gramicidin. Consistent with these findings, whole cell patch clamp studies have demonstrated an increase in membrane conductance with 0.5X Ringer's that was inhibited by quinine and H-7. Further, 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-9303427.)

59.8

IONIC EQUILIBRIA IN SHARK RED CELLS.

J.A. Payne and T.J. McManus. Mount Desert Island Biological Laboratory, Salsbury Cove, ME, 04672, Dept. of Zoology, Univ. of Florida, Gainesville, FL 32611 and Division of Physiology, Department 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 nurse shark, *Ginglymostoma cirratum*, a warm water form. In both species, distribution ratios of Cl (r_{Cl}) and H (r_H) ions varied inversely with the external pH (pH_o) and were equivalent to each other over the physiological range (7.0-8.4), as predicted by Donnan theory. Moreover, when pH_o was varied and the membrane potential estimated by the indirect method of Macey et al. (BBA 512: 284-295, 1978), it correlated strongly with r_{Cl}. Taken together, these results support the assumption that Cl and H are at equilibrium at pH_o>7.0 in cells from both species. However, at pH_o<7.0, a marked difference between r_{Cl} and r_H is apparent. The proton ratio becomes greater than the chloride ratio as pH_o falls, an effect that is most significant in dogfish red cells. Evidence to be presented suggests that this disequilibrium is the result of an acid-induced stimulation of Na/H exchange, as well as the increasing inhibition of the Jacobs-Stewart cycle at low pH_o. The actual values of the distribution ratios of Cl and H ions were much lower that those reported for red cells from most other vertebrate species. They were also relatively insensitive to changes in pH_o. These two findings can be explained by the high osmolality of elasmobranch extracellular fluids, and the abundance of impermeant cell organic solutes required to offset this osmolality. When Na and K permeability was increased by application of gramicidin (1μM) to nurse shark red cells, r_{Na} and r_K became equal to each other, but much greater than r_H at all values of pH_o. Moreover, when plotted against pH_o, r_{Na} and r_K do not cross the r_{Cl} line at a value of 1.0, as seen in human red cells (Payne et al. AJP 259: C819-C827, 1988). This apparent disequilibrium of Na and K in a cation permeabilized cell may reflect a reduction of cytosolic cation activity owing to the presence of stabilizing methylamines, such as TMAO, betaine and sarcosine, thought to be necessary to offset the effects of high urea concentration on enzymes and other intracellular proteins.

59.9

VOLUME REGULATION IN TWO FRESHWATER MOLLUSCS, LAMPSILIS TERES AND POMACEA BRIDGESI, IN HYPEROSMOTIC MEDIA.

Percy J. Jordan and Lewis E. Deaton. Dept. of Biology, University of Southwestern Louisiana, Lafayette, LA 70504.

L. teres and P. bridgesi were acclimated to media ranging from fresh water (5-9 mOsm) to 393 mOsm and from fresh water (FW) to 151 mOsm, respectively. L. teres survied in media above 200 mOsm while P. bridgesi died inmedia 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 amino acid content increased from 14.86 ± 4.69 umol/g dry weight (FW) to 315.75 ± 125.94 umol/g dry weight (393 mOsm). B-alanine, glycine, and alanine were the major constituents of the free amino acid pool at all external osmotic concentrations. In P. bridgesi gill, the amino 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 molluscs 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

EFFECT OF EXTERNAL ACIDIFICATION ON CALCIFICATION IN THE FRESHWATER BIVALVE *Anodonta imbecilis*. Dazhong Xu* and Michele G. Wheatly. Univ. of Florida, Gainesville, FL 32611

In neutral water unidirectional Ca influx and efflux decrease with bivalve body mass. Smaller animals tended to lose Ca while larger animals remain in Ca balance. Unidirectional Ca influx followed enzyme-saturation kinetics. The half saturation [Ca] was 0.213 mM. The maximum unidirectional Ca influx rate was 4.329 µmol/g dry mass/h. External [Ca] had no effect on unidirectional Ca efflux. Gill had the highest Ca content followed by the digestive gland, mantle and foot. There was a linear correlation between tissue ^{45}Ca accumulation and [Ca]. *A. imbecilis* recommences ventilation shortly after immersion in acid experimental water (pH 4.0, H_2SO_4) making it a good model for studying the effect of acid on calcification. Severe acid exposure (pH = 5.2, adjusted every 2h) inhibited the unidirectional Ca influx (37.1%) and increased unidirectional efflux (87.3%) resulting in net Ca efflux (increased 443%). ^{45}Ca accumulation decreased in EPF (39.8%), inner surface of the shell (52.8%), mantle (66.6%), foot (63.9%), gill (59.9%) and digestive gland (75.2%); [Ca] of EPF increased (19.9%) and there were decreases in [Ca] of mantle (43.3%), foot (51.6%) and digestive gland (46.0%), but not gill. A lower ^{45}Ca specific activity in tissues than in the medium (24h) suggests that tissue Ca is largely non-exchangeable. Less severe acid exposure (pH = 4, not adjusted) caused a net loss of Ca. Exposure to AW for 7 and 60 days did not affect the [Ca] of EPF or tissues. (Supported by NSF 89-16412).

59.11

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 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 ion-conformed in the most highly concentrated media. Rates of unidirectional Na^+ influx and combined flux (influx and efflux) exhibited a significant positive correlation with acclimation salinity. 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

TRANSEPITHELIAL POTENTIAL DIFFERENCE AND SODIUM FLUXES ACROSS ISOLATED PERFUSED GILLS OF THE HYPER-HYPOREGULATING MANGROVE CRAB ***Ucides cordatus***. Cláudia B. R. Martinez*[1], Robert R. Harris[2] and Maria do Carmo F. Santos*[3].[1] Universidade Estadual de Londrina, Londrina, Pr., 86050-970, Brasil; [2] University of Leicester, Leicester, LE17RH, England; [3] Universidade de São Paulo, São Paulo,S.P., 05499, Brasil.

Transepithelial Potential Differences (TEP) and unidirecional sodium fluxes were determined in ***Ucides*** posterior gills (numbers 5 and 6) perfused, internally, with specific saline (750 mOsm/KgH_2O), which closely matched the composition of the crab hemolymph, and, externally, with 9,17,26 and 34S (S = ppt salinity). In the symetrical condition (26S), TEP were small and similar for both gills (-0.08 ± 0.25 and -0.11 ± 0.16). In all others salinities TEP exhibited opposite polarities and varied differently in response to the modification of the NaCl level in the incubation medium. This could indicate different types of ion movements in gill 5 and 6. Rates of sodium influx determined in gill 5 varied from 726.93 ± 103.52 to 1111.47 ± 135.55 µMNa^+/g.h and sodium efflux from 337.49 ± 56.49 to 689.03 ± 108.87µMNa^+/g.h, influx rates were larger than efflux rates in all experimental salinities. In relation to gill number 6 influx rates were much smaller and varied from 3.67 ± 0.25 to 8.45 ± 0.79 µMNa^+/g.h, while efflux rates increased significantly in response to dilution of the external medium and varied from 87.24 ± 20.25 to 223.27 ± 19.38 µMNa^+/g.h. Flux ratios of experimentally obtained values for gill 5 were slightly higher than those predicted by Ussing's criterion in all experimental conditions. In the case of gill 6, experimentally determined flux ratios were much smaller than predicted values in all salinities. These results showed functional differences between the two pairs of gills, indicating number 5 gill as a site for sodium uptake while gill 6 is probably for sodium extrusion. Both gills, acting together, play a important role related to the strong osmoregulatory capacity showed by this mangrove crab.

Research supported by CAPES - Brasil

59.13

MECHANISMS OF KCL REABSORPTION IN THE LOWER MALPIGHIAN TUBULE OF *RHODNIUS PROLIXUS*. Charlene Haley and Michael J. O'Donnell, Department of Biology, McMaster University, Hamilton, Ont. L8S 4K1

The Malpighian tubules of the blood-feeding insect, *Rhodnius prolixus*, consist of a secretory upper portion, which transports KCl luminally, and a reabsorptive lower portion. Reabsorption of KCl in the lower tubule can be stimulated *in vitro* by 5-HT. Following stimulation, the lower tubule can reduce luminal fluid osmolality from 370 to 250 mOsm kg^{-1}. This is primarily caused by a reduction in luminal [K^+] from as high as 70 mM to less than 5mM. In this study, drugs known to inhibit related ion-transport pathways were used to block KCl reabsorption in the lower tubule. Three experimental techniques were employed. The first approach was to use whole isolated tubules *in vitro* with the upper and lower portions separately bathed in saline droplets under paraffin oil. The second approach involved perfusion of drugs through the lower tubule following cannulation. For these procedures, ion-selective microelectrodes were used to measure [K^+] in fluid secreted from the lower tubule and thus % inhibition of reabsorption. The final technique employed involved measurement of basolateral membrane potentials during perfusion of the whole, isolated tubule with specific drugs. Present research has revealed evidence for an apically-sited Cl^-/HCO_3^- exchanger. Drugs known to block Cl^-/HCO_3^- exchangers, such as diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS), 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid (SITS), and Acetazolamide (an inhibitor of epithelial carbonic anhydrase and hence internal bicarbonate production), inhibited KCl reabsorption. A number of chloride channel blockers, including N-phenylanthranilic acid (DPC), 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), and NaSCN, inhibited KCl reabsorption; rapid inhibition suggests localization of Cl^- channels on the basolateral membrane. Imidazopyridine SCH 28080, a reversible inhibitor of gastric H^+/K^+-ATPase also blocked potassium reabsorption, and may serve in pH regulation. Inhibition of K^+ reabsorption by barium indicates a role of potassium channels, although their localization remains unknown.

59.14

PEPTIDE INCREASES PARACELLULAR PERMEABILITY IN INSECT MALPIGHIAN TUBULE. Klaus W. Beyenbach and Ari B. Rubenfeld. Section Physiology, Cornell University, Ithaca, N.Y. 14853.

The diuretic leucokinin, an octapeptide isolated from cockroach heads (Holman et al., Comp. Biochem. Physiol. 88: 31, 1987), dramatically reduces transepithelial voltage and resistance in isolated perfused Malpighian tubules of the yellow fever mosquitoe *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 leucokinin on transcellular or paracellular pathways, we measured transepithelial permeabilities of inulin. The permeability to inulin was of interest because inulin is excluded from movement through transcellular pathways. Hence transepithelial permeabilities reflect the properties of an extracellular pathway such as the paracellular pathway. Leucokinin (1µM) significantly increased the rate of transepithelial fluid secretion (1.98-fold) and significantly increased transepithelial inulin permeability (2.26-fold; 11 Malpighian tubules). The diuretic cAMP (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 cAMP confirm its action on the transcellular pathway impermeable to inulin (Sawyer & Beyenbach, Am. J. Physiol. 248: R339, 1985). Since transepithelial inulin permeability increases in the presence of leucokinin but not cAMP, the effect of leucokinin on the paracellular pathways is demonstrated. Supported by NSF IBN 9220464.

59.15

OSMOREGULATION IN THE CRAYFISH (PROCAMBARUS CLARKII): HORMONAL REGULATION BY ATRIAL NATRIURETIC PEPTIDE. Carl L. Reiber and Michele G. Wheatly. University of Nevada, Las Vegas, NV 89154

Atrial natriuretic peptide has proven to be a multi-functional hormone in mammalian systems, with natriuretic, diuretic and vasoactive actions. Mammalian studies indicated ANP release 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. Injections of ANP at 10^{-13} M concentration increased both Na^+ and Cl^- influx significantly. Ion efflux rate did not match influx resulting in a net positive influx, as indicated by an increase in hemolymph ion concentration. Na^+/K^+-ATPase activity decreased significantly in the antennal glands and gut with a threshold response of 10^{-13} M ANP. Gill Na^+/K^+-ATPase activity increased only slightly at 10^{-10} M ANP. Guanylate cyclase activity in crayfish gills is activated by ANP in a dose dependant manner. Cyclic GMP concentration increases in gill membrane preparation when exposed to ANP concentrations as low as 10^{-9} M. Freshwater crayfish are presented with the same challenge to salt and water homeostasis as freshwater fish. Modulation of osmoregulatory functions by ANP results in an increase in ion influx at the gill and decrease ion loss via the gut and antennal glands (urinary ion output). This would be of adaptive advantage for crustaceans exposed to an hypoosmotic environment or during ecdysis.

59.16

HYPERTHERMIC AND HYPERTONIC SHOCK INDUCE HSP70 ACCUMULATION AND THERMOTOLERANCE BUT NOT OSMOTIC TOLERANCE IN CULTURED MAMMALIAN RENAL CELLS. Paul H. Yancey* and Lance P. Walsh* Whitman College, Walla Walla, Wa. 99362

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 (Madin-Darby canine kidney; MDCK) cells (Cohen et al., AJP 261:C594, 1991). To test if hsp70 can protect cells from osmotic shock, we treated MDCK cells to heat shock (45°C), osmotic shock (590 mosm with added NaCl), or combination (initial shock, 2-6 hrs recovery at 37°C and 300 mosm, then final shock). We judged cell survival by cell plating efficiency and by total protein in culture flasks grown 24 hr with cells surviving experimental treatment; both gave similar results. Hsp70 was detected by monoclonal antibody (Western blots, chemiluminescent detection). First, a 10-min heat shock enhanced thermotolerance if cells were given 6 hr of recovery, which maximized hsp70 accumulation (Combination = 10 min shock, 6 hr recovery, 35 min shock; survival results are % of controls ± SD, hsp results are relative blot intensities):

	Control	Heat shock 10 min	Heat shock 35 min	Combination
Protein:	100 ± 3	67 ± 10	7 ± 1	73 ± 8
Hsp70:	-	+	+	+++

Second, we confirmed that osmotic shock induces hsp70, which continued to build up for 2 hr recovery but disappeared by 4 hr. We then found that the presence of hsp70--whether induced by an initial heat or osmotic shock--did not improve survival in a later osmotic shock (Combination 1 = 1 hr osmotic shock, 2 hr recovery, 24 hr osmotic shock Combination 2 = 10 min heat shock, 6 hr recovery, 24 hr osmotic shock):

	Control	Heat shock 10 min	Osmotic shock 1 hr	Osmotic shock 24 hr	Combination 1	Combination 2
Protein	100 ± 3	61 ± 1	63 ± 3	30 ± 3	29 ± 2	24 ± 6
Hsp70:	-	+	+	+	+	+++

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59.17

NA,K-ATPASE ALPHA ISOFORM EXPRESSION IN FIVE RAT SKELETAL MUSCLES. Curtis B. Thompson* and Alicia A. McDonough. Dept. Physiology, Univ. Southern Calif. Med. Sch., L. A., CA 90033

The sodium pump (Na,K-ATPase - NKA) is a heterodimer of a catalytic α subunit and a glycosylated β subunit. Three isoforms of each have been identified. We have previously shown isoform specific ionic and hormonal regulation of NKA in gastrocnemius muscle where α2 and β2, not α1 or β1 are regulated by hypokalemia and thyroid hormone. We aimed to determine relative levels of expression of α isoforms in a panel of skeletal muscles: Soleus (Sol), Red Gastrocnemius (RG), Extensor Digitorum Longus (EDL), White Gastrocnemius (WG), and Diaphragm (Dia). These muscles are representative of fiber types Type I (I), Type IIA (IIA), and Type IIB (IIB) approximately as follows: Sol - 87% I; RG - 30% I, 62% IIA; EDL - 50% IIA, 50% IIB; WG - >85% IIB; and Dia - heterogeneous mix I, IIA, IIB. Relative abundance of α1, α2 and α3 were determined via immunoblot analysis with α specific antibodies(Ab). Data was normalized to diaphragm. α1 and α2 were expressed in all fibers. α3, previously reported only in neonatal rat muscle, was expressed in all five muscles at very low levels. α1 and α2 levels ranked in order from greatest to least, were as follows, α1: Dia>Sol>RG>EDL>WG; α2: Dia>Sol,EDL>RG>WG. α1 expression was 17 fold higher in Dia than in WG, and α2 was expressed 4 fold higher in Dia than WG. Thus, α isoform type appears independent of muscle type, with levels differing significantly between muscle types. 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 α2β2 regulation in muscle; including the redistribution of K stores in hypokalemia, and thyroid thermogenesis.

59.18

MOLECULAR IDENTIFICATION OF A P-TYPE ATPASE FROM THE GENOME OF *Coccolithus pelagicus*: IMPLICATIONS FOR MOLECULAR EVOLUTION AND CELL BIOLOGY. Yan Song* and Douglas Fambrough, Biology Department, the Johns Hopkins Univeristy, Baltimore, Maryland 21218

Using polymerase chain reaction amplification, we have isolated a DNA fragment from total genomic DNA of *Coccolithus pelagicus*, which belongs to the marine phytoplanktonic 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 FSBA binding site. The new clone's sequence contains the FITC-binding site, located about midway between the two primers. All three sites are definitive domains on the P-type cation-motive ATPases. The sequence of this coccolithophorid ion pump most resembles the protozoan and fungal intracellular Ca^{2+}-ATPases, the mammalian Cu^{2+} and secretory pathway Ca^{2+}-ATPase, the prokaryote Mg^{2+}- and Cd^{2+}-ATPases and the plasma membrane H^+-ATPases in fungi and higher plants. This DNA sequence is among the first P-type pumps cloned from a free-living protist, which may shed new light on the molecular evolutionary history of the P-type ATPases. Meanwhile, a possible secretory pathway cation pump in *Coccolithus* may represent a substantial step towards a fuller understanding of the processes invovled in cellular calcification as exemplified by this species.

59.19

ONTOGENY OF OSMOREGULATION IN THE EMBRYOS OF *GAMMARUS DUEBENI* LILLEJEBORG (CRUSTACEA: AMPHIPODA: GAMMARIDAE). **David Morritt & John I. Spicer , Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2UQ, U.K.**

The osmoregulatory ability of eggs and embryos of the gammarid amphipod *Gammarus duebeni* , exposed to a range of external concentrations (75 - 1250 mOsm.kg^{-1} at 15° C), was examined. For all the stages examined the isosmotic point was similar (500-550 mOsm kg^{-1}). Periembryonic fluid (PF - fluid from the space between the embryo and the vitelline membrane) from early stage 2 and 3 embryos (darkening cephalothorax and limb bud development) and haemolymph from new hatchlings exhibited the same hyper-isosmotic regulation pattern as present in adult haemolymph. Maintenance of a hyperosmotic PF establishes the necessary osmotic gradient for the uptake of water which causes the characteristic swelling of embryos. It is evident that PF osmolality is regulated in dilute external concentrations before the ontogeny of the coxal gills, possibly by the vitelline membrane and/or the dorsal organ. In contrast, PF from later, stage 5-7 embryos (eye pigmentation and cardiovascular development but still pre-hatching) showed a distinct hyper-hypo-osmotic pattern of regulation. The functional significance of this hyper-hypo-osmoregulatory pattern is unknown although it's appearance may be associated with the development of the coxal gills and the concomitant degeneration of the dorsal organ.This study has demonstrated that osmoregulatory function changes with ontogeny in amphipods and that the most complicated pattern (hyper-hypo-regulation) is associated with late embryo rather than the most complex (adult) stage.

59.20

THE ONTOGENY OF OSMOTIC TOLERANCES IN THE AMERICAN SHAD (*Alosa sapidissima*). Joseph Zydlewski* and 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 ontogeny of salinity tolerance has been widely studied in salmonid species, but is largely unknown for other anadromous species. This is especially true for the juvenile American shad (*Alosa sapidissima*). Such information is required to judge the significance of delays in downstream passage caused by dams. The ontogeny and mechanism of salinity tolerance of this commercially important fish were studied. Adult shad from the Connecticut River were artificially spawned and the young were reared in the lab. Bi-weekly 35 ppt seawater challenges indicate that the onset seawater tolerance coincides with metamorphosis, reaching 100% survival between 45-58 days post-hatch. Whereas salmonids develop salinity tolerance during the parr-smolt transformation just prior to and during migration, American shad juveniles establish tolerance 3 months prior to the peak of migration. Juvenile shad introduced into high salinity exhibited a characteristic increase in gill Na^+,K^+-ATPase activity. A time series sampling of migrating wild juveniles in the Connecticut River showed a gradual 2.5 fold increase in gill Na^+,K^+- ATPase activity while plasma chloride ion content showed a 21% gradual decrease. Seawater acclimated post-migratory juveniles were unable to osmoregulate in freshwater (< 1 ppt) in isothermal transfer or through acclimation over a two week period. It is postulated that the increase in gill Na^+,K^+-ATPase activity is related to the loss of ions which may act as a proximate cue for the seaward migration.