T-Pos197 EFFECTS OF 2,3-BUTANEDIONE MONOXIME (BDM) ON FORCE PRODUCTION AND MYOSIN LIGHT CHAIN PHOSPHORYLATION (MYLCP) IN MAMMALIAN SMOOTH AND SKELETAL MUSCLE. T.B. Warren, T.M. Butler, M.J. Siegman and S.U. Mooers, Dept. of Physiology, Thomas Jefferson Univ., Phila., PA 19107.

It has been suggested (Mulieri and Alpert, Biophys. J. 45:47a, 1984) that BDM selectively blocks crossbridge interaction and associated ATP utilization in muscle. We have tested the effects of BDM on rabbit taenia coli and mouse EDL. In the electrically stimulated smooth muscle, BDM causes a proportional decrease in both active force output and MYLCP in a dose-dependent manner (ED_{50}=7 mM). The actions of BDM could not be reversed by increasing $[Ca^{++}]_o$ from 1.9 to 4.5 mM. BDM may thus block contraction in smooth muscle by preventing MYLCP. In contrast to the effects on smooth muscle, BDM decreased force output to a greater extent than MYLCP in skeletal muscle. The ED_{50} for force is 2 mM. In 15 mM BDM, where force is less than 15% P_o, MYLCP in response to a 7 sec tetanus is inhibited by only 40% and multiple tetani resulted in phosphorylation of over 75% of the myosin light chains. Therefore, myosin light chain kinase is activated under conditions when actin-myosin interaction, as measured by active force production, does not occur. A preliminary estimate of the force-dependent energy usage was obtained by direct measurement of high-energy phosphate utilization in mouse EDL at L_o in which force was graded using 0-15 mM BDM. The force-dependent energy usage is 45% of the total energy usage in a 7 sec tetanus. This is in good agreement with previous estimates in which force was changed by variation in filament overlap (J. Mus. Res. Cell Motil. 5:45, 1984). Thus, in skeletal muscle BDM may decrease force with little change in the energy requirements of activation processes, and it appears to interfere with crossbridge cycling by a different mechanism than it does in smooth muscle. (Supported by HL15835 to the Pennsylvania Muscle Inst. and RCDA AM 973 to TMB)

T-Pos198 AN IMPROVED TECHNIQUE FOR THE PREPARATION OF CHEMICALLY SKINNED SMOOTH MUSCLE, Joe R. Haeberle, Judith A. Tanner, and Jeffrey W. Hott, Depts Physiology and Biophysics, Indiana Univ. Sch. Med., Indianapolis, IN, 46223.

Mechanically stable rat uterine and porcine carotid skinned smooth muscles have been prepared utilizing glycerination in the presence of a thiol-protease inhibitor. Pieces of intact tissues are equilibrated in a physiologic saline solution (PSS) containing no calcium, 1 mM EGTA, and 1 mM leupeptin for 1 h at room temperature. The undissected tissues are then transferred to the skinning solution which contains: 40 mM Imidazole, 5 mM EGTA, 6 mM MgATP, 1 mM Mg^{2+}, 1 mM DTT, and 0.5 mM leupeptin. The muscles are skinned in this solution for 48 h at 5°C, transferred to fresh skinning solution, and frozen at -70°C. Immediately prior to use, the muscles are thawed and a thin layer of muscle (25-100 um thick) is dissected from the surface of the tissue. Muscles prepared in this manner have undergone repeated contraction/relaxation sequences (6-8) over a period of 3 h with less than a 10% reduction in isometric force and less than a 10% increase in resting force. The stoichiometry of phosphorylation of the 20,000 dalton myosin light chain (LC20) was 0.52 $\pm$ 0.09 mol PO_4/mol LC20 for the uterine muscle and 1.0 mol PO_4/mol LC20 for the carotid muscle in the presence of 30 uM calcium and 10 uM calmodulin. In the presence of thio-ATP, both muscles were phosphorylated to 1.0 mol PO_4/mol LC20. Electron microscopic examination of both tissues demonstrated normal myofilament arrays within individual cells and little or no tissue damage (i.e. loss of cytoskeletal and myofilamentous structures) along the dissected edge of the tissue. This work was supported by grants from the American Heart Association, Indiana Affiliate; the NIH (HL 06308); and the Herman C. Krannert Fund.

T-Pos199 MYOSIN PHOSPHATASE: EFFECT ON GIZZARD SKINNED FIBERS. Phyllis E. Hoar, Mary D. Pato+, and W. Glenn L. Kerrick; Dept. of Physiology and Biophysics, Univ. of Miami, Miami, FL.: +Dept. of Biochemistry, Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Skinned cells of chicken gizzard were used to study the effect of a smooth muscle phosphatase (SMP-IV) on activation and relaxation of tension. SMP-IV has previously been shown to dephosphorylate light chains on myosin. When this phosphatase was added to submaximally Ca^{2+}-activated skinned cells, tension increased while phosphorylation of myosin light chains decreased. In contrast when SMP-IV was added to cell bundles activated in the absence of Ca^{2+} by a Ca^{2+}-insensitive myosin light chain kinase, tension and phosphorylation of the myosin light chains both decreased. These data suggest that Ca^{2+} inhibits the deactivation of tension even when myosin light chains are dephosphorylated to a low level. Furthermore, comparison of Ca^{2+}-activated cells caused to relax in CTP, in the presence or absence of Ca^{2+}, shows that cells in the presence of Ca^{2+} do not relax completely, whereas in the absence of Ca^{2+} cells completely relax. Solutions containing Ca^{2+} and CTP however, are incapable of generating tension from the resting state. Endogenous myosin light chain kinase is not active in solutions containing CTP, and dephosphorylation of myosin light chains occurs in CTP solutions both in the presence and absence of Ca^{2+}. These data imply that Ca^{2+} inhibits relaxation even though myosin light chains are dephosphorylated. These data are consistent with a model wherein an obligatory Ca^{2+}-activated myosin light chain phosphorylation is followed by a second Ca^{2+} activation process for further tension development or maintenance. Supported by American Heart Association, Muscular Dystrophy Association and Medical Research Council of Canada and Saskatchewan Health and Resources Board.

T-Pos200 THE EFFECTS OF NUCLEOSIDE DIPHOSPHATE AND INORGANIC PHOSPHATE ON TENSION IN SKINNED SOLEUS AND SMOOTH MUSCLE CELLS. W.G.L. Kerrick & P.E. Hoar, Dept. of Physiology & Biophysics, Univ. of Miami, Miami, FL.

The products of actomyosin ATPase, nucleoside diphosphate and inorganic phosphate (Pi) have been found to affect tension in skinned soleus and smooth muscle cells. All solutions contained 2 mM $MgATP^{2-}$, 7 mM EGTA, 85 mM K^+, 1 mM Mg^{2+}, 10^{-9}-$10^{-3.8}$ M Ca^{2+}, and imidazole propionate to maintain pH=7.0 and ionic strength of 0.15. In addition $MgADP^-$ or Pi were incorporated into the test solutions. In rabbit soleus 10 mM Pi decreased maximum Ca^{2+}-activated tension by 22% and shifted the pCa for 50% maximum tension from 5.73 in control solutions to pCa 5.18 for Pi solutions. In contrast 5 mM MgADP increased maximum tension by 11% and shifted the pCa for 50% maximum tension from the control to pCa 6.77. MgADP concentrations above 2 mM also caused tension to develop in relaxing solutions (pCa=9.0). CDP, IDP, UDP and GDP affected tension in a similar manner to ADP. In contrast, in smooth muscle both maximal Ca^{2+}-activated tension and maximal tension activated by myosin light chain thiophosphorylation in the absence of Ca^{2+} were not affected by MgADP. Inorganic phosphate (10 mM) decreased maximum tension in smooth muscle as it did in the soleus. [MgADP] above 5 mM caused tension to increase in relaxed smooth muscle cells at pCa=9.0. The results are consistent with a kinetic model of muscle contraction suggesting that regulation involves the release of inorganic phosphate and tension generation is associated with a myosin-ADP complex. These results also suggest that the Ca^{2+} affinity of the regulatory proteins is affected by the kinetics of actomyosin ATPase. Supported by grants from the American Heart Association and Muscular Dystrophy Association.

T-Pos201 LENGTH-DEPENDENCE OF Ca^{2+} SENSITIVITY AND STRESS MAINTENANCE IN SKINNED SWINE CAROTID MEDIA. Robert S. Moreland and R. A. Murphy. Dept. of Physiology, Univ. of Va. Sch. Med., Charlottesville, VA 22908

Ca^{2+}-dependent stress development was proportional to myosin light chain phosphorylation (MLCP) in skinned carotid media. However, stress was maintained when Ca^{2+} was reduced to levels that did not support phosphorylation (latch) (Chatterjee and Murphy, Science 221: 464, 1983). The latch state was expressed only after initial MLCP and/or stress development. This study was designed to examine the influence of developed stress on stress maintenance and latch. Tissues were mounted at 0.7, 1.0, or 1.4 L_o, where L_o = length for maximal active stress. Strips were skinned with Triton X-100, exposed to solutions of increasing (phosphorylation and stress development) or decreasing (dephosphorylation with stress maintenance) [Ca^{2+}].

Measurement (units)	Length (L/L_o) 0.7	1.0	1.4
Maximum Stress (x 10^4 N/m^2)	2.2 ± 0.2*	7.1 ± 0.2	4.5 ± 0.4*
K_m for Stress Development (μM Ca^{2+})	1.7 ± 0.4	1.4 ± 0.3	3.0 ± 0.3*
K_m for Stress Maintenance (μM Ca^{2+})	0.42 ± 0.05	0.54 ± 0.03	2.6 ± 0.6*
K_m for Myosin Phosphorylation (μM Ca^{2+})	1.6 ± 0.2	1.5 ± 0.1	1.0 ± 0.3

[Means ± 1 SEM, n = 5 - 9, * = different from values at L_o, $p < 0.05$.] The results show that stress *per se* had no effect on the Ca^{2+}-dependent regulatory mechanisms because stress at $L_o > 1.4\ L_o > 0.7\ L_o$. However, increased muscle length and perhaps decreased filament overlap shifted the calcium-dependence curve for stress development to the right, with no effect on the K_m for MLCP. Furthermore, latch was abolished at 1.4 L_o [with or without 1 μM calmodulin]. Supported by NIH grants 5P01 HL19242 and F32 HL06532.

T-Pos202 MEASUREMENT OF MAXIMUM UNLOADED SHORTENING VELOCITY IN SINGLE ISOLATED SMOOTH MUSCLE CELLS. Shinobu Yagi and Fredric S. Fay, Physiol., U. of Mass. Med. Sch., Worcester, MA

The kinetics of virtually all aspects of smooth muscle contraction are believed to be considerably slower than striated muscle. While both V_{max} and the actomyosin (AM) ATPase rates are depressed in smooth muscle relative to striated muscle, the extent of depression in V_{max} is much greater than that of ATPase rates. To determine whether this difference indicates a fundamental difference in the limiting factor for AM and cross-bridge turnover between smooth and striated muscles or results from uncertainties in the V_{max} obtained on strips of tissue which are quite complex, we undertook an investigation of contractile kinetics of unloaded shortening of single isolated smooth muscle cells (SMC's). SMC's isolated by enzymatically disaggregating slices of stomach muscularis from *Bufo marinus*, were mounted for isometric measurement of force and subjected to rapid releases to varying extents (10-30% of cell length, L_i) beyond their slack length. Maximum unloaded shortening velocity was determined from analysis of the time required to develop force following releases In each cell, 3-5 releases were performed after force had plateaued following electrical stimulation. The time to just redevelop forces was linearly related to the extent of releases. Unloaded shortening velocity was determined from the slope of this relation. In contrast to results from multicellular preparations, our data revealed unloaded shortening velocity between 0.5-1.0 L_i/sec (0.74 ± 0.13 (SE) n=10), several times greater than previously reported. The relation between this V_{max} and the activites of smooth muscle AM ATPase corresponds well with that (V_{max}/ATPase) of striated muscle. This is consistent with the notion that the steps limiting AM ATPase and V_{max} are probably similar in smooth and striated muscles. Supported by grants from NIH and MDA. S.Y. is an MDA postdoctoral fellow.

T-Pos203 CHANGES IN MYOSIN LIGHT CHAIN PHOSPHORYLATION DURING HISTAMINE-INDUCED RHYTHMIC CONTRACTION OF HOG CAROTID ARTERY SMOOTH MUSCLE. S.P. Driska, R. Porter, and P.G. Stein. Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298

Stimulation of hog carotid artery smooth muscle strips with submaximal doses (10 uM) of histamine causes rhythmic contractions with peak forces up to 90% of those developed at higher histamine concentrations (which cause tonic contractions). Between contractions, in the continued presence of histamine, the strips relax to as little as 10% of the peak force. This allowed us to study changes in myosin light chain phosphorylation (LCP) in response to muscle activation without complications due to agonist diffusion. Seven muscle strips were prepared from each of four different arteries and stimulated with histamine. After development of rhythmic contractions, they were frozen with dry ice - acetone at various points in their contraction-relaxation cycles. The peak force represented a mean active stress of $1.57 \pm 0.32 \times 10^5$ N/m^2 (SD, N=26). Between contractions these tissues relaxed to 28 ± 8 % of peak force (SD, N=26). LCP was determined by isoelectric focusing/SDS-electrophoresis and expressed as a percentage (± SEM) of the total 20,000 dalton light chain. LCP was lowest in relaxed muscles after histamine washout (13 ± 5 %). LCP was slightly (but not significantly) higher in the presence of histamine between contractions (20 ±3%). Higher levels of phosphorylation were found near the time when peak force was reached: 32 ± 6 % at half of peak force, contracting; 47 ± 5 % at the peak of force; and 22 ± 9 % at half of peak force, relaxing. Under these conditions, the highest level of phosphorylation was not reached before the maximum force was reached. This is unlike the case where force development is initiated by addition of an agonist to the bathing solution. Supported by NIH HL24881 and HL01198.

T-Pos204 CHANGES IN RATE OF CROSSBRIDGE CYCLING DURING CONSECUTIVE TETANI IN SMOOTH MUSCLE. M.J. Siegman, T.M. Butler and S.U. Mooers, Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pa., 19107 USA

We have examined force output, chemical energy usage ($\Delta\sim P$) and myosin light chain phosphorylation (MYLCP) during a double tetanus paradigm in the rabbit taenia coli at 20°C in order to find the basis for a marked reduction in the rate of force redevelopment during the second tetanus. Following a 25sec isometric tetanus and 30sec of relaxation, a second tetanus of 25sec duration was initiated. Compared to the first tetanus, 90% force redeveloped with a proportional change in dynamic stiffness; maximum shortening velocity (Vmax) was markedly reduced and MYLCP was 60% lower; the average rate of $\Delta\sim P$ for force redevelopment was about ½ that for initial development of force and not different from the $\Delta\sim P$ associated with maximum force maintenance during a single tetanus (J. Gen. Physiol.76:609, 1980). These results during the second tetanus could be due to (a) a slower intrinsic rate of crossbridge cycling, or (b) resistance to shortening presented by dephosphorylated "latch-bridges." The $\Delta\sim P$/work during the second tetanus is not significantly different than that measured during the first. These similar efficiencies for work output argue against the presence of "latch-bridges" which would be expected to decrease efficiency. Rather, there seems to be a general slowing of the rate of crossbridge cycling *per se*. This might be due to less calcium release during the second tetanus, resulting in a lower degree of MYLCP and slower cycling of phosphorylated cross-bridges, reflecting a direct regulatory effect of calcium on Vmax (Pflugers Arch.401:385, 1984) with eventual redevelopment of force. The results predict that following initial activation of smooth muscles in vivo, the energy demands for continued phasic contractions in a population of cells should be very low. Supported by HL15835 to the Penna Muscle Institute and RCDA K04 AM00973 to T.M.B.

T-Pos205 IS THERE HYSTERESIS IN SMOOTH MUSCLE CALCIUM-TENSION RELATIONSHIPS? K.G. Morgan and T.T. DeFeo*. Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

Previous reports have suggested that chemically skinned vascular smooth muscle (VSM) displays hysteresis in its calcium-tension relationships ($[Ca^{2+}]_i$-T). We have attempted to use aequorin-loaded intact VSM preparations to confirm these findings. Strips of either ferret portal vein (FPV) or ferret aorta (FA) were loaded with the bioluminescent Ca^{2+} indicator aequorin by a previously described chemical loading procedure (Morgan & Morgan, J Phys 351:155,1984). Graded K^+-depolarizations were used to induce changes in $[Ca^{2+}]_i$ which were measured as changes in emitted light. Steady state light signals were calibrated by the fractional luminescence method of Allen & Blinks (Nature 273;509,1978) and were plotted against steady state tension for both an increasing and a decreasing order of K^+-depolarization. If $[Mg^{2+}]_i$ is assumed to be 0.5mM, threshold $[Ca^{2+}]_i$ for the initiation of tone is 2.0×10^{-7}M and 1.6×10^{-7}M; and $[Ca^{2+}]_i$ during a maximally effective (66mM) K contracture is 3.7×10^{-7}M and 3.8×10^{-7}M in FPV and FA, respectively, at 35°C. Thus, $[Ca^{2+}]_i$-T curves from these intact cells, as indicated by aequorin, were steeper than is usually reported for skinnned preparations. We were unable to demonstrate any hysteresis in the $[Ca^{2+}]_i$-T curve for FPV but FA consistently showed a leftward shift after exposure to a high $[Ca^{2+}]_i$. These data make it unlikely that the previously described increased sensitivity of the contractile apparatus to $[Ca^{2+}]_i$ in the presence of phenylephrine in FPV is due to a hysteresis-related phenomenon. The difference in the behavior of FPV and FA may be related to the more "phasic" nature of FPV contractions compared to the slower more "tonic" contractions of FA. Support: HL31704 and an Am. Heart Grant-in-Aid and Established Investigatorship to KGM.

T-Pos206 PROSTAGLANDIN-MEDIATED CHANGES IN $[Ca^{2+}]_i$ SENSITIVITY IN CORONARY ARTERY SMOOTH MUSCLE. A.B. Bradley*, T.T. DeFeo*, and K.G. Morgan (Intr. by J.P. Morgan). Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

The aim of this study was to determine the effect of $PGF2_\alpha$ on $[Ca^{2+}]_i$ and force (F) in hog coronary arteries (hca). Using hca strips loaded with aequorin, we have previously reported that the aequorin light (L) (i.e. $[Ca^{2+}]_i$) responses of carbachol (Carb) and serotonin (5-HT) consist of an initial L spike followed by a fall in L to a lower plateau level during continued force maintenance. We have now found that $PGF2_\alpha$ causes a parallel monophasic rise in F and L without an initial L spike. In contrast to Carb- and 5-HT - induced contractions that were characterized by a $[Ca^{2+}]_i$ spike, the rate of rise of force during $PGF2_\alpha$ contractions was significantly slower. Comparison of $PGF2_\alpha$, Carb, and 5-HT responses with K^+ "control" contractions showed that the F/L ratios for comparable forces at steady state were higher than for K^+. Furthermore, after 20 minutes of hca exposure to a Ca^{2+}-free EGTA medium, which lowered resting hca F and L, addition of $PGF2_\alpha$ initiated a sustained tonic hca contraction with no detectable change in $[Ca^{2+}]_i$. -- These data indicate: 1) that PGF_α contracts hca largely by an increase in the Ca^{2+} sensitivity of the contractile apparatus; 2) that the $PGF2_\alpha$ mediated rise in $[Ca^{2+}]_i$ sensitivity occurs without previous exposure to high $[Ca^{2+}]_i$ and is thus not a manifestation of $[Ca^{2+}]_i$ - tension hysteresis; 3) that the initial $[Ca^{2+}]_i$ spike seen in contractions elicited by other receptor agonists such as Carb and 5-HT may play a role in accelerating the rate of hca force development. Support:HL31704, AHA Grant in Aid, and Established Investigatorship to KGM; and HL06748 to ABB.

T-Pos207 THE INFLUENCE OF VARYING INTRACELLULAR MAGNESIUM LEVELS ON VASCULAR SMOOTH MUSCLE CONTRACTILITY by George D. Ford and Steven P. Driska, Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298.

Numerous studies have shown that variations in extracellular magnesium influence the contractile response of vascular smooth muscle (VSM) to a wide variety of agonists. However little is known about the possible influence of intracellular magnesium on the contractility of VSM and if this could, in part, account for the observed influence of extracellular magnesium. In these studies, porcine carotid arterial strips were incubated for up to four hours in a high K^+ (123.8mM), zero Ca^{2+} medium with varying magnesium levels (0-30mM) and with and without 1μM ouabain to acutely induce changes in intracellular magnesium levels. The response to any agonist was virtually abolished by magnesium depletion, i.e.; 4 hr incubation with 0 Mg. The response to high K^+ exhibited a sigmoid relationship with respect to the [Mg] in the incubation medium, with no effect demonstratable following incubation with at least 15 mM $MgCl_2$. 50% of the preincubation response was obtained following incubation with 3mM $MgCl_2$. At low levels of Mg incubation (up to 1.2mM), the dose-response curve for intracellular Ca^{2+}-dependent norepinephrine (NE) responses was shifted to the right and depressed, while at higher Mg levels there was a decreased response to low and high levels of agonist but a nearly equal response to intermediate levels of agonist. However the dose-response curves to NE using normal extracellular Ca levels tended to only exhibit a decreased maximum response. These results suggest intracellular Mg levels may alter excitation-contraction coupling processes as well as contractile protein function. (Supported by a grant-in-aid from the American Heart Association and HL-24881.)

T-Pos208 CALCIUM-DEPENDENCE OF MYOSIN DEPHOSPHORYLATION DURING RELAXATION OF TRACHEAL SMOOTH MUSCLE. William T. Gerthoffer. Dept. Pharmacology, Univ. Nevada School of Med., Reno, NV 89557.

Dephosphorylation of myosin precedes relaxation of arterial smooth muscle (Gerthoffer and Murphy, Amer J. Physiol. 245: C271, 1983), but coincides with the more rapid relaxation of rabbit tracheal muscle (Gerthoffer and Murphy, Amer. J. Physiol. 244: C182, 1983). We predicted that dephosphorylation would precede relaxation during a slow relaxation of tracheal muscle if myosin dephosphorylation is not the rate limiting step. This hypothesis was tested by comparing the rate of relaxation of canine tracheal muscle to the rates of myosin dephosphorylation and the decay of isotonic shortening velocity. Muscles were contracted with 10^{-6} M carbachol for 5 min, and relaxed by: (i) Washing out the carbachol, or (ii) Exposure to Ca^{2+} free-physiological salt solution containing 0.1 mM EGTA and carbachol. Relaxation was rapid during agonist washout (t1/2 - 0.7 min), and the initial rates of relaxation, myosin dephosphorylation and decay of shortening velocity were similar. Relaxation was slower following Ca^{2+} removal (t1/2 - 2.8 min), and the rate of dephosphorylation and decay of shortening velocity were both dissociated from relaxation. Shortening velocity decayed more rapidly than the muscle relaxed, but myosin dephosphorylated more slowly than the muscle relaxed. Phosphorylation decreased from 0.63 (± 0.06) to 0.46 (± 0.03) moles Pi/mole light chain after 4 min., at which time active stress was 46 (± 9) % of the initial value. The Ca^{2+}-dependence of crossbridge inactivation appears to be different from the Ca^{2+}-dependence of myosin dephosphorylation in tracheal muscle, and Ca^{2+} clearance may not be the sole factor regulating dephosphorylation following muscarinic activation. (Supported by Research Advisory Board, Univ. Nevada-Reno and Amer. Heart Assoc.-Nev. Affiliate).

T-Pos209 EFFECTS OF CA^{2+} AND MG^{2+} ON MYOSIN AND SHORTENING VELOCITY OF GIZZARD SMOOTH MUSCLE. R. Barsotti, M. Ikebe and D. Hartshorne, Muscle Biol. Grp., Univ. of Az., Tucson, AZ 85721.

The existence of a Ca^{2+}-dependent regulatory mechanism in smooth muscle in addition to myosin phosphorylation has been suggested. Our studies investigate this possibility in gizzard muscle. The following was found: a Ca^{2+}-dependence of actin-activated ATPase activity of phosphorylated myosin that was due primarily to a reduction in the absence of Ca^{2+} of the apparent affinity for actin: Ca^{2+}-binding to myosin to a maximum of 2 mol Ca^{2+}/mol myosin; a Ca^{2+}-dependent shift in the KCl-dependence of myosin viscosity; an enhanced sensitivity to papain digestion of phosphorylated myosin in the presence of Ca^{2+}; a tendency for oligomer formation of phosphorylated myosin in the presence of Ca^{2+}. In general, these effects were seen at only low free Mg^{2+} concentrations and were lost at 3-4 mM free Mg^{2+}. (The relationship between the Mg^{2+} concentration and the Ca^{2+} sensitivity of ATPase activity has been emphasized by Chacko and colleagues.) The slack test was used to examine the effects of Ca^{2+} and Mg^{2+} on the unloaded shortening velocity (Vus) of skinned gizzard fibers. In the presence of 5 mM $MgCl_2$, 5 mM ATP and 1 uM calmodulin the ratio of developed force at 0.3 uM and 16 uM Ca^{2+} was 0.94 $\pm$ 0.05 (N=10), while the ratio of Vus at these Ca^{2+} levels decreased to 0.68 + 0.06 (N=10). The extent of myosin phosphorylation at the two Ca^{2+} concentrations was similar (42 $\pm$ 4%, N=8, and 40 $\pm$ 2%, N=6, respectively). At 7 mM $MgCl_2$, 5 mM ATP, 1 uM calmodulin Vus at 3 uM Ca^{2+} increased, and the ratio's of developed force and Vus at the two Ca^{2+} levels were the same ($\sim$ 1). The extent of myosin phosphorylation also was similar. These data suggest that under some conditions Ca^{2+} can modulate the ATPase activity of phosphorylated myosin and the cross-bridge cycling rate. Supported by grants HL 23615 and 20984 from NIH and an MDA fellowship to R. B.

T-Pos210 **METABOLIC COMPARTMENTATION IN VASCULAR SMOOTH MUSCLE (VSM): EVIDENCE FOR AT LEAST TWO FUNCTIONALLY INDEPENDENT POOLS OF GLUCOSE-6-PHOSPHATE. Ronald M. Lynch and Richard J. Paul, Univ. of Cincinnati, Dept. of Physiology & Biophysics, College of Medicine, Cincinnati, OH 45267-0576**

We have shown that the substantial aerobic lactate production in VSM is correlated with the Na-pump while oxidative metabolism is more strongly related to isometric force. Furthermore, even though glycogenolysis is substantial, lactate is produced solely from medium glucose (Science 222:1324, 1983). To account for these findings, two separate glycolytic pathways must be operable within the VSM cell. In this study, we measured the concentration and specific activity (sa) of glucose-6-phosphate (G-6-P) to further characterize this compartmentation. Measurements of sa were made on porcine carotid arteries which were incubated in a Krebs solution containing U-[^{14}C]-glucose. A purified G-6-P fraction was obtained using a Dowex-1-Cl column and a potassium-borate gradient. Tissue G-6-P content was 0.027±.002 µmol/g (n=19). G-6-P radiolabel attained a steady state within 30 min, at a specific activity ratio (G-6-P) sa/(glucose) sa of 0.83± 0.02 (n=7). This indicates that there exists a pool of G-6-P which is not readily accessible to extracellular glucose. Contraction was then elicited by the addition of KCl (80 mM). Glycogenolysis was rapid over the first 30 min, amounting to approximately 1.2 µmol/g. Intracellular [G-6-P] was unaltered after 30 min, however, the G-6-P sa ratio decreased to a value of 0.68±0.01 (n=3). These data indicate a dilution of the G-6-P sa due to addition of relatively unlabeled G-6-P derived from glycogen into the total G-6-P pool. Since the lactate sa was found to be unaltered, these data confirm that there are at least two functionally separate glycolytic pathways, one for the utilization of medium glucose with lactate as the end-product, and another involved in glycogenolysis. Supported by NIH 23240 and an Established Investigatorship (RJP) of the American Heart Association.

T-Pos211 FORCE:VELOCITY RELATIONSHIP IN SINGLE ISOLATED SMOOTH MUSCLE CELLS. David M. Warshaw, Department of Physiology & Biophysics, University of Vermont, Burlington, VT 05405

Previous tension transient studies in single smooth muscle cells (SMCs) (Warshaw and Fay, J. Gen. Physiol. 82:157, 1983) suggest that smooth muscle's slow, economical contractile capabilities reflect differences in both crossbridge elasticity and kinetics of the crossbridge cycle. To gain further insight into crossbridge cycle kinetics, studies to characterize the steady state force:velocity relationship (F:V) were performed in single SMCs. Single SMCs were enzymatically isolated from the toad (Bufo marinus) stomach muscularis and attached to a force transducer and length displacement device. F:V were obtained at the peak of isometric contraction (Fmax) by setting active cell force to a new level (0.05-0.90Fmax) using feedback control and recording the resultant shortening of cell length (Lcell) in response to the force step. Using a linearized form of the Hill equation, F:V in single SMCs at 20°C were characterized by a maximum shortening velocity (Vmax) of 0.20Lcell/s, with constants a/Fmax=0.17 and b=0.03Lcell/s. To verify Vmax calculated from F:V, Vmax were obtained using the slack test method and were found to be in agreement (Vmax=0.19 ± 0.09Lcell/s, x + se, n=4) . These mechanical data confirm that low Vmax in smooth muscle is an inherent cellular property. In addition, F:V data can be interpreted in terms of crossbridge population distributions and cycle kinetics to help explain SMCs slow, economical contraction.

T-Pos212 ENERGETICS OF WORKING CONTRACTION AT LOW EXTRACELLULAR CALCIUM $[Ca^{2+}]_o$ IN PORCINE CAROTID ARTERY. Joseph M. Krisanda and Richard J. Paul, Department of Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

We have demonstrated that the cost of maintaining isometric force in vascular smooth muscle (VSM) is not a constant, but increases with increasing $[Ca^{2+}]_o$. This observation may result from a direct effect of $[Ca^{2+}]_o$ on the efficiency of the chemomechanical transduction process. Recently we reported a Fenn effect in VSM with an efficiency that is much less than that observed in striated muscle. The purpose of our current investigation was to determine whether efficiency in VSM changes as a function of $[Ca^{2+}]_o$. Strips (n=5) of porcine carotid artery were incubated in MOPS-buffered PSS (0.15 mM $[Ca^{2+}]_o$) and stimulated isometrically with KCl (+50 mM) at 37°C. The unloaded shortening velocity (V_{us}) and isometric force, measured after 10 min of stimulation, were 0.008 + 0.002 l_o/s and 52 ± 13 mN/mm , 1.9 and 2.4 times lower than values collected using 2.5 mM $[Ca^{2+}]_o$. After 10 min of stimulation, phosphagen breakdown ($\Delta\sim P$) was measured following a 10 s isovelocity shortening at 0.28 V_{us} for maximum power output. The work output was 1.0 ± 0.3 mJ/g, 2.4 times less than that at 2.5 mM $[Ca^{2+}]_o$. Tissue ATP and PCr did not significantly differ from control values, and $\Delta\sim P$, calculated from steady-state measurements of J_{O_2}, was 0.035 μmol/g. Work per $\Delta\sim P$ was 28.9 ± 9.4 kJ/mol, which when corrected for work due to the series and parallel elastic elements yielded an active work per $\Delta\sim P$ of 5.4 ± 2.7 kJ/mol. These data indicate that at low $[Ca^{2+}]_o$ a Fenn effect is not observed in VSM. Additionally, although the efficiency is approximately two-fold higher at low $[Ca^{2+}]_o$, this value is still substantially less than that observed for striated muscle.

Supported by NIH grant HL23240 and a grant from the American Heart Association.

T-Pos213 BARR, LLOYD, and GU, FANJI, The Photomechanical Resposne of the Frog Iris, Department of Physiology and Biophysics, University of Ilinois, Urbana, IL 61801.

Iridial sphincter pupillae from a wide variety of vertebrates, from eels to hamsters, contract when light is shined on them. This response is apparently triggered by the absorption of light by rhodopsin resident in the smooth muscle cell membrane. We are testing the hypothesis that bleaching this rhodopsin leads directly to a release of internally stored calcium and that in turn the rise of free Ca^{++}, via a calmodulin-myosin light chain kinase cascade, activates the mechanical apparatus. We have applied a variety of pharmacological agents to irises during 'in vitro' experiments in order to interfere with specific purported steps in our hypothetical schema. Agents we found to inhibit the photomechanical response are trifluoperzine, chlorpromizine, cAMP 8Bromo-cAMP, 8Benzylamino-cAMP, forskolin, isobutylmethylxanthine, theophylline, papaverine, adenosine and isoproterenol. Agents which did not inhibit are verapamil, TTX, cGMP, 8Bromo-cGMP, 8dibutryl cAMP or cGMP and phenylepherine. Using "reasonable" estimates of the kinetic parameters of our hypothetical schema we have been able to simulate the time courses of the photomechanical responses.

T-Pos214 EVIDENCE FOR SMOOTH MUSCLE MYOSIN ISOZYMES. Pagani ED, Faris R, Shemin R*, Julian FJ. Depts of Anesthesia Res & *Cardiac and Thoracic Surg, Brigham & Women's Hospital, Boston, MA.

Takano-Ohmuro et al. (J Biochem 93:930, 1983) reported that smooth muscle from embryonic chicken gizzard contains three myosin isozymes. Here we present evidence to suggest that adult mammalian smooth muscle may also contain myosin isozymes. Tissue rich in smooth muscle (rabbit: aorta, intestine, uterus; human: aorta, saphenous vein) or media dissected from rabbit aorta or human saphenous vein was homogenized, within 5 to 120 minutes after dissection, in 100mM pyrophosphate buffer (pH 8.8 at $4^{o}C$) containing 5 mM EGTA, 10% glycerol and proteolytic enzyme inhibitors. The myosin was extracted for 14 to 20 hours at $4^{o}C$ then immediately electrophoresed on pyrophosphate gels as previously described (Circ Res 54: 586, 1984); however, the gels were silver-stained using a modification of the method of Morressey (1981). Tissue and media extracts yielded two bands on pyrophosphate gels. The bands were sliced from the gels and analyzed by SDS gel electrophoresis. Each band was composed of a high molecular weight protein (heavy chain) and two low molecular weight proteins (light chains) of 20,000 and 17,000 daltons. Segments of the media from a rabbit aorta were either made to contract by exposure to 10 uM epinephrine or kept relaxed, immediately frozen in liquid nitrogen and then homogenized in NaF-pyrophosphate extraction solution (Silver and Stull, J Biol Chem 257 (11) 1982). Extracts from contracted and relaxed smooth muscle yielded two bands having the same mobility and staining intensity on pyrophosphate gels. Thus under our conditions, the second myosin band is not a result of myosin phosphorylation. These data suggest that two myosin isozymes may be present in rabbit and human smooth muscles. Supported by NIH Grants HL06563 (EDP) and HL30133 (FJJ).

T-Pos215 REACTION OF CHICKEN GIZZARD MYOSIN WITH PHENYLGLYOXAL. Gary Bailin, UMDNJ-School of Osteopathic Med., Piscataway, NJ 08854

Chicken gizzard myosin incorporated nearly 4 mol of phenyl [2-^{14}C]-glyoxal per 4.7 x 10^{5}g of protein with a slight change in the K^{+}-ATPase activity in the presence of EDTA. When 3 additional mol of the reagent were incorporated the K^{+}-ATPase activity was inhibited. Phenylglyoxal reacted with arginine residues of gizzard myosin in a mol ratio of two to one, phenylglyoxal to arginine. The modification was limited to the heavy chain region and none of the light chains were lost. The phenylglyoxal groups were located in the subfragment 1 and in the rod-like regions of gizzard myosin but major changes in the ATPase activity occurred when the subfragment 1 region was modified predominantly. Essentially the same results were obtained when the myosin was pretreated with the myosin light chain kinase calcium-calmodulin phosphorylating system and then incubated with phenylglyoxal. Substrate $MgATP^{2-}$ enhanced the inactivation of gizzard myosin; there was an increase in the incorporation of the reagent but no change in the distribution in the heavy chains. Approx. 0.5 mol of the nucleotide was bound per 4.7 x 10^{5}g of phenylglyoxal myosin. The lack of stoichiometric trapping of ATP indicates that conformational changes, induced by these modifications, were responsible for the inhibition of enzymic activity. Pepsin digests of phenylglyoxal [2-^{14}C] gizzard myosin with and without ATP yielded similar elution patterns. Two major peptide fractions contained at least 70% of the total radioactivity recovered. These peptides correspond to the subfragment 1 region and part of the rod-like region of the heavy chains of gizzard myosin. Arginine residues of gizzard myosin are necessary for the maintenance of its ATPase activity. Supported, in part, by American Heart Assoc. (N.J. Aff.).

T-Pos216 ACTIVATION OF THE ATPase OF PHOSPHORYLATED GIZZARD MYOSIN BY GIZZARD ACTIN: EFFECTS OF Ca^{2+}, Mg^{2+} AND TROPOMYOSIN, Hidetake Miyata* and Samuel Chacko; Department of Pathobiology, University of Pennsylvania, Philadelphia, PA

Actin-activation of gizzard myosin is dependent on Ca^{2+} and Mg^{2+} concentration even after stable phosphorylation (Heaslip and Chacko Biophys J. 45: 1984). Tropomyosin potentiates the actin-activated ATP hydrolysis and the potentiated activity is 50-80% higher in the presence of Ca^{2+} at free Mg^{2+} concentration of about 2 mM. Experiments were carried out to determine if the increased activity in the presence of Ca^{2+} is due to a Ca^{2+}-mediated binding of tropomyosin to actin which causes an increase in the tropomyosin potentiation of actin-activated ATP hydrolysis. Purified gizzard tropomyosin iodinated using [^{125}I] was incubated with gizzard actin (Tm:A molar ratio = 1:6) at ionic conditions and protein concentrations identical to those used for ATPase assays. The Ca^{2+} concentration was kept either at pCa8 or at pCa4; the free Mg^{2+} ion varied from 0.5 to 8 mM. The binding of tropomyosin to gizzard F-actin is dependent on Mg^{2+} both in the presence and absence of Ca^{2+}. At 0.5 mM Mg^{2+} tropomyosin binding in the absence of Ca^{2+} was 3 fold higher than in the presence of Ca^{2+}. Upon increasing the Mg^{2+} to 8 mM, the binding was raised to 60% at pCa8 and to 55% at pCa4. At free Mg concentration of 2 mM, a concentration at which the maximum Ca^{2+} activation of actin-activated ATPase was observed, tropomyosin bound to actin was slightly higher at pCa8. The binding study and ATPase assay were also performed at 2 mM free Mg^{2+} and varying pCa. These experiments showed that the tropomyosin binding was slightly higher at lower Ca^{2+} concentrations. The correlation between ATPase activity and tropomyosin binding indicates that the Ca^{2+} stimulation of actin-activated ATP hydrolysis is not due to a Ca^{2+}-mediated binding of tropomyosin to actin. Supported by HL 22264 and PCM-8309139. H.M. is an MDA post-doctoral fellow.

T-Pos217 THE EXTENT OF REGULATION OF THE MgATPase ACTIVITY OF SMOOTH MUSCLE HEAVY MEROMYOSIN IS REVEALED BY SINGLE-TURNOVER EXPERIMENTS. James R. Sellers, Laboratory of Molecular Cardiology, NHLBI, NIH, Bethesda, MD 20205.

Phosphorylation of smooth muscle heavy meromyosin (HMM) has been shown to result in a 25-fold increase in the steady-state V_{max} of the actin-activated MgATPase activity from 0.07 s^{-1} for dephosphorylated HMM (dePHMM) to 1.8 s^{-1} for phosphorylated HMM (PHMM) (Sellers et al. J. Biol. Chem. 257, 13880, 1982). The steady-state MgATPase activity of dePHMM in the absence of actin is 0.004 s^{-1}. The true extent of regulation might be even larger since the actin-activation of the MgATPase activity of dePHMM (from 0.004 s^{-1} to 0.07 s^{-1} at V_{max}) could be arising from a small fraction of modified HMM molecules which are no longer regulated and that truly regulated dePHMM molecules are not activated by actin. To test this idea a "limited turnover" experiment (Wells and Bagshaw, FEBS Lett. 168, 260, 1984) was used to measure the reassociation rate of acto-dePHMM following addition of a two-fold molar excess of ATP. The reassociation (as measured by turbidity) was initially fast, followed by a predominant slow phase. The fast phase probably represents the reassociation with actin of some more active unregulated HMM molecules after they have hydrolyzed all of the free ATP. The rate of the slow phase was not significantly increased by raising the actin concentration from 10 μM to 75 μM and was estimated to be about 0.002 s^{-1}, which is in good agreement with the rate of product (both P_i and ADP) release from dePHMM alone measured by a gel filtration technique. These two experiments suggest that the rate of product release from dePHMM may not be significantly affected by actin and that perhaps the true extent of regulation of HMM by phosphorylation is much greater than that determined by steady-state methods.

T-Pos218 EFFECT OF PHOSPHORYLATION ON THE BINDING OF SMOOTH MUSCLE MYOSIN·ADP TO ACTIN by Lois Greene and Jim Sellers. LCB, NHLBI and LMC, NHLBI, NIH, Bethesda, MD 20205.

Relaxation of both smooth and skeletal muscles appears to be caused primarily by inhibition of a kinetic step in the actomyosin ATPase cycle, the step associated with Pi release, rather than by a block in the binding of the myosin·ATP and myosin·ADP·Pi complexes to actin. In skeletal muscle, troponin-tropomyosin not only causes marked inhibition of Pi release, but it also inhibits the binding of S-1·ADP to actin, raising the possibility that the two phenomena are coupled in some way. In the present study we determined whether dephosphorylation of smooth muscle HMM also affects both the Pi release step and the binding of HMM·ADP to actin. This was done by competing phosphorylated and nonphosphorylated HMM for sites on F-actin. In contrast to skeletal muscle, the binding of smooth HMM to actin in the presence of ADP was not significantly inhibited as part of the regulatory process. At μ = 30 mM, dephosphorylation only decreases by ~4 fold the affinity of each head of HMM for actin (~12-fold for the molecule), while at μ = 170 mM, there was less than a 2-fold decrease in the affinity of each head. At both ionic strengths, there is marked inhibition of the actin-activated ATPase activity of smooth HMM by dephosphorylation. Therefore, the inhibition of Pi release with smooth acto·HMM, which could be as large as 900-fold (Sellers, Biophys. Abst. 1985), is not accompanied by inhibition of the binding of myosin·ADP to actin. We have also found that dephosphorylation decreases by only 3-fold the rate of Pi release from HMM alone. These results suggest that in smooth muscle, dephosphorylation inhibits the step associated with the release of Pi both in the forward and the reverse direction and this inhibition occurs without a corresponding effect on the binding of M·ADP to actin.

T-Pos219 THE HEAVY CHAIN OF MACROPHAGE MYOSIN IS PHOSPHORYLATED AT THE TIP OF THE TAIL J.A.Trotter, C.Nixon and M.Johnson, Dept. of Anatomy, University of New Mexico School of Medicine, Albuquerque, N.M. 87131

Rabbit alveolar macrophage myosin is phosphorylated in intact cells on the 20kDa light chain and on the heavy chain (B.B.R.C. 106:1071, 1982). Analysis of the amount of inorganic phosphate bound to the heavy chain, isolated from DEAE-purified myosin, has yielded stoichiometries ranging from 0.44 to 0.89 mol Pi/mol HC (5 separate preparations). Thin layer electrophoresis following partial acid hydrolysis of ^{32}P-phosphorylated heavy chain shows phosphoserine as the only phosphoamino acid. Two-dimensional peptide mapping of ^{32}P-phosphorylated heavy chain digested with trypsin shows a single constant radioactive spot, together with several weaker spots which vary from experiment to experiment in terms of their number, positions and relative intensities. These results suggest that each heavy chain is phosphorylated at a single site. Digestion of insoluble ^{32}P-phosphorylated myosin with papain yields a soluble fragment which binds F-actin in an ATP-reversible manner, and an insoluble fragment. All the heavy-chain radioactivity is associated with the insoluble fragment. Limited tryptic digestion of ^{32}P-phosphorylated myosin in solution (0.5M KCl) simultaneously removes all radioactivity from the heavy chain and reduces its apparent MW by less than 10kDa. Taken together, these observations suggest that the heavy chain is phosphorylated within 10kDa of the -COOH terminus (the tip of the tail), and that this phosphorylation may involve a single serine residue.

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T-Pos220 DICTYOSTELIUM CHEMOTAXIS: EXTRACELLULAR CYCLIC AMP TRIGGERS THE IN VIVO PHOSPHORYLATION OF MYOSIN. C.H. Berlot*, P.N. Devreotes+, and J.A. Spudich*, *Dept. of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305, and +Dept. of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

When D. discoideum amoebae are starved, they develop the ability to perform chemotaxis towards cAMP. cAMP stimulation of chemotactically competent amoebae labeled with ^{32}P transiently increased phosphorylation in both the heavy chain and the 18 kD light chain of myosin as determined by immunoprecipitation, SDS-PAGE, and autoradiography. The increases in phosphorylation peaked at 30 sec and returned to the baseline by ~ 3 min. These kinetics closely parallel a shape-change response in amoebae exposed to a temporal jump in cAMP concentration (D. Fontana et al. (1984) in The Cell Surface in Development and Cancer, Plenum Pub.) The dose-dependency of the phosphorylation response corresponds well with that of chemotaxis (P. Van Haastert (1983) J. Biol. Chem. 258:9643). These phosphorylation increases occured even when cAMP-induced activation of adenylate cyclase was blocked by pre-treatment of amoebae with caffeine. Phosphorylation increases in the myosin heavy chain were also observed when unlabeled amoebae were stimulated with cAMP, then lysed into a reaction mix containing $\gamma[^{32}P]$-ATP. When excess myosin was added to this mix, phosphorylation increases were observed in the light chain as well. The kinetics of the in vitro phosphorylation responses are sufficient to predict the time-course of the in vivo response, assuming that it is caused by kinase activation, with little or no modulation of phosphatase activity. In support of this, assays of myosin phosphatase in cell lysates revealed a low level of activity that did not change during the response. (Supported by NIH grant #GM25240 to JAS.)

T-Pos221 ACTIN ACTIVATION OF BRUSH BORDER MYOSIN Mg^{2+} ATPASE ACTIVITY BY A CALCIUM-INDEPENDENT KINASE. Borysenko, C., Collins, H., Lamperski, B., Montibeller, J., Rieker, J. and Collins, J.; Dept. of Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15216.

The brush border of intestinal epithelial cells is a widely studied model system for non-muscle contractile proteins. Contraction of the circumferential ring of microfilaments attached to the zonula adherens in the terminal web region of isolated brush borders is accompanied by the phosphorylation of myosin. We show here that highly purified brush border myosin can be phosphorylated on both its heavy chains and its 20,000-dalton light chains by a calcium independent kinase-containing fraction from brush border. Phosphorylation to about 2 mol of phosphate/mol of myosin results in the conversion from an essentially non actin-activatable state to a form activated about 20-fold by actin with a specific activity 0.54 μmol/min/mg. Preliminary results with peptide mapping of complete tryptic and chymotryptic digests of phosphorylated heavy chain and LC_{20} indicate that phosphorylation occurs at more than one site on each of these chains. Most of the phosphorylation occurs at a threonine residue(s) on the LC_{20}. A small but significant amount of phosphorylation also occurs at a threonine residue(s) in heavy chain, and in trace amounts at a serine residue(s) in LC_{20}. Partial purification of the kinase using gel filtration, high performance anion exchange and heparin-sepharose chromatography was achieved. We are currently attempting to determine which phosphorylation site(s) regulates actin-activation of ATPase activity and further characterize the kinase. We also present a new, rapid purification procedure used to obtain brush border myosin that is based on gel filtration and high performance anion exchange chromatography.

T-Pos222 ANTIBODIES TO SPECIFIC REGIONS OF THE TAIL OF ACANTHAMOEBA MYOSIN II ACT AS PROBES OF THE REGULATION OF Mg^{2+} ATPase ACTIVITY. Mark A.L. Atkinson, Graham P. Côté, Ettore Appella, and Edward D. Korn, LCB, NHLBI and LCB, NCI, National Institutes of Health, Bethesda, MD 20205.

Myosin II from Acanthamoeba castellanii has an actin-activated Mg^{2+} ATPase activity which is regulated by phosphorylation of 3 serine residues on the carboxyl terminus of the molecule. 58 residues, including the phosphorylation sites, have been sequenced in this region; the sequence of amino acids is N-S-A-L-E-S-D-K-Q-I^{10}-L-E-D-E-I-G-D-L-H-E^{20}-K-N-K-Q-L-Q-A-K-I-A^{30}-Q-L-Q-D-E-I-D-G-T-P^{40}-S-S-R-G-G-S-T-R-G-A^{50}-S-A-R-G-A-S-V-R (Cote et al. (1984) J. Biol. Chem. 259, 12781-12787). The first 36 residues of this peptide are predicted to form a coiled-coil, a structure characteristic of the tail regions of other myosins. Beyond residue 36 the helix is broken and the characteristic hydrophobic repeat of a coiled-coil is lost, suggesting that this terminal region is in either a random coil or globular conformation. The 3 phosphorylatable serines are found within this region at residues 46, 51 and 56. We have synthesized two peptides in this region of the molecule. One contains residues 11 to 30 in the coiled-coil region and the other residues 39-58 in the random/globular region. Using these peptides cross-linked to BSA as antigens we have raised polyclonal antibodies. These antibodies bind to the intact myosin and their respective peptides in an ELISA assay and to the myosin in a Western blot. The antibodies to the phosphorylatable peptide bind much more strongly to dephosphorylated myosin than phosphorylated myosin. Following affinity purification, we have used these antibodies to investigate how antibody binding to these two adjacent, structually distinct regions in the tail affect the Mg^{2+} ATPase activities of the head.

T-Pos223 EFFECT OF ACTIN FILAMENT LENGTH ON THE ACTIN-ACTIVATED ATPase OF MYOSIN FROM ACANTHAMOEBA. J.P. Albanesi, M. Coué, H. Fujisaki and E.D. Korn (Intr. by S. Brenner, DuPont Experimental Station, Wilmington, DE), NHLBI, NIH, Bethesda, MD 20205

Myosins IA and IB from Acanthamoeba castellanii share the enzymatic properties of other muscle and non-muscle myosins but have lower molecular weights and show no tendency to form filaments. When phosphorylated on their heavy chains, their actin-activated Mg^{2+}-ATPase activities are increased about 50-fold. Recently we have shown that the actin-activated ATPase activity can be expressed in either a cooperative or a non-cooperative fashion. At low myosin:actin ratios (approx. 1:200), the specific activity is independent of myosin concentration and follows a classic hyperbolic dependence upon actin concentration. At higher myosin:actin ratios the specific activity increases cooperatively with myosin concentration until a plateau is reached. We have studied the effect of actin filament length on both the non-cooperative and cooperative actomyosin I activity states. With plasma gelsolin, a barbed end capping protein, we were able to obtain actin filaments of different lengths by varying the gelsolin:actin ratio at a constant F-actin concentration. We find that in the presence of short filaments (gelsolin:actin ratios greater than 1:200), higher myosin concentrations are required to reach the cooperative state. The extent of this shift to higher myosin requirement is directly proportional to the gelsolin:actin ratio. In addition, short filaments obtained with gelsolin:actin ratios lower than 1:10 were more effective in activating the non-cooperative actomyosin activity state than was the normal F-actin.

T-Pos224 PURIFICATION AND CHARACTERIZATION OF LOW MOLECULAR WEIGHT MYOSINS FROM DICTYOSTELIUM. G.P. Côté, J.P. Albanesi, T. Ueno and E.D. Korn, NIH, NHLBI, Bethesda, MD 20205.

Acanthamoeba castellanii contains two low-molecular weight, single-headed myosins, IA and IB, with heavy chains of 130 kDa and 125 kDa, respectively, in addition to conventional myosin II. When phosphorylated on their heavy chains by a specific Acanthamoeba myosin I kinase, the actin-activated Mg^{2+}ATPase activities of myosins IA and IB increase approximately 50-fold. Two similar low-molecular weight myosins, with heavy chains of 125 kDa and 120 kDa, have now been separated from the conventional Dictyostelium myosin by chromatography on DE-52, and purified by precipitation with F-actin and affinity adsorption on ADP-agarose. The 120-kDa protein is monomeric by sedimentation equilibrium. By several additional criteria, the small Dictyostelium myosins are similar to those from Acanthamoeba and unlike the conventional Dictyostelium myosin (heavy chains, 250 kDa) or its active chymotryptic fragment (heavy chain 130 kDa): the Acanthamoeba and Dictyostelium small myosins have high (K^+,EDTA)-ATPase activities (approx. 4 $\mu mol\ min^{-1}\ mg^{-1}$); both Dictyostelium small myosins react with antibodies to Acanthamoeba myosin I, by the Western-blot technique; and both Dictyostelium small myosins are phosphorylated by Acanthamoeba myosin I heavy chain kinase. The 120 kDa Dictyostelium myosin is a better substrate for Acanthamoeba kinase and its actin-activated Mg^{2+}-ATPase activity is enhanced 10-fold by phosphorylation. The conventional Dictyostelium myosin and its chymotryptic derivative have low (K^+EDTA)-ATPase activities, do not react with antibodies to Acanthamoeba myosin I, and are not phosphorylated by Acanthamoeba myosin I kinase.

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T-Pos225 SUPERPRECIPITATION OF NON-FILAMENTOUS ACANTHAMOEBA MYOSIN I WITH SKELETAL MUSCLE F-ACTIN. H. Fujisaki, J.P. Albanesi, T.J. Lynch and E.D. Korn, NIH, NHLBI, Bethesda, MD 20205.

Since Szent-Gyorgyi first presented superprecipitation of actomyosin solution as an in vitro model of muscle contraction, formation of myosin filaments has been believed to be essential for superprecipitation; neither heavy meromyosin or myosin subfragment-1, which are unable to assemble into filaments, cause superprecipitation. Acanthamoeba myosins IA and IB are single-headed globular molecules that do not form filaments. Nevertheless, we have now shown that myosin I causes superprecipitation with rabbit skeletal F-actin in the presence of Mg^{2+}-ATP. Experiments were carried out under the following conditions: 2 mM ATP, 2 mM $MgCl_2$, 14 mM KCl, 0.1 mM $CaCl_2$, 6 mM imidazole, 0.3 mM DTT, 0.01% NaN_3, and 7% glycerol at pH 7.5 and 25°C. In microcapillaries, birefringent actomyosin threads were formed. The formation of a contractile actomyosin gel in a spectrophotometer cuvette was quantified by the increase in absorbance at 660 nm. Phosphorylation of the myosin I heavy chain was required for superprecipitation as well as for actin-activated Mg^{2+}-ATPase activity. With phosphorylated myosin I, superprecipitation required Mg ions and ATP. The rate and extent of the absorbance change were dependent on the concentrations of myosin I and F-actin. With 0.3 and 0.6 μM myosin IA, maximum superprecipitation was observed at 2 and 4 μM F-actin, respectively; i.e. a molar ratio of myosin I to actin subunits of 1:7. Under these conditions, absorbance increased immediately upon the addition of Mg^{2+}-ATP (no clearing phase) and ATP hydrolysis was linear throughout the course of experiments. These observations are consistent with our previous hypothesis, from other data, that myosin I contains two F-actin-binding sites or undergoes F-actin-induced oligomerization.

T-Pos226 ISOLATION OF NON-MUSCLE MYOSIN GENES FROM ACANTHAMOEBA. J.A. Hammer III, E.D. Korn, and B.M. Paterson. LCB, NHLBI, and LB, NCI, National Institutes of Health, Bethesda, MD 20205

We are interested in isolating the myosin heavy chain genes of the soil amoeba Acanthamoeba castellanii with the goal of analyzing myosin structure/function relationships utilizing site-directed mutagenesis. Acanthamoeba simultaneously express three distinct myosin enzymes, myosin IA, myosin IB, and myosin II. We have used a portion of the nematode C. elegans myosin heavy chain gene (gift of J. Karn, MRC, Cambridge), which encodes the actin and ATP binding sites and the active thiols, to search for Acanthamoeba myosin heavy chain genes. We screened a genomic library of Acanthamoeba DNA in phage lambda with this heterologous myosin probe and obtained approximately 4 positive phage per genome equivalent. Restriction mapping of 16 clones yielded 3 groups of overlapping phage and several individual non-overlapping phage. One phage group was tentatively identified as containing an amoeba myosin II heavy chain gene based on the following observations: the phage DNA hybrid selected a mRNA which translated in vitro to yield a 185 kDa protein; the 185 kDa protein co-migrated in SDS-PAGE with authentic, purified myosin II heavy chain, and was quantitatively and specifically immunoprecipitated by myosin II antiserum; and the phage DNA insert hybridized to a 5300 bp amoeba mRNA. We used a mixed 20 bp oligonucleotide probe synthesized from a portion of 58 amino acid residues sequenced protein chemically from a C-terminal myosin II peptide (Côté et al, JBC, 259, 12781, 1984) to locate the 3' end of the gene. Sequencing of a DNA restriction fragment which hybridized to this probe revealed a stretch of nucleotides encoding the 58 amino acids, confirming the identity of the myosin II gene. We are currently analyzing other clones for the presence of myosin I genes.

T-Pos227 LOCATION AND REACTIVITY OF THIOLS OF THE GIZZARD MYOSIN HEAVY CHAIN Sumitra Nag, Narindar Nath, and John C. Seidel, Dept. of Muscle Res., Boston Biomed. Res. Inst., Boston, MA

Modification by MalNEt of two thiols, one (SH-A) in the myosin rod and one (SH-B) in the 17k light chain, is accompanied by changes in ATPase activity and in the 6S-10S conformational transition, which mimic those seen on phosphorylation of the 20k light chain (Chandra et al., J. Biol. Chem., in press). Myosin labeled at SH-A with ^{14}C-MalNEt was digested with papain and 80% of the protein-bound ^{14}C was in a 120k peptide of the rod. On chymotryptic digestion, 80% of the ^{14}C of myosin appears in heavy meromyosin and 90% of the ^{14}C of the rod appears in subfragment-2, locating SH-A in this region. K^+-EDTA ATPase activity (K-ATPase) is inhibited by modification of SH-C, the inhibition being substantially accelerated by ATP. When the SH-C thiols were labeled with ^{14}C-MalNEt and the myosin digested with papain, ^{14}C was found in the 95, 70 and 25k peptides of subfragment-1. ATP produced a 3- to 4-fold increase in the incorporation of ^{14}C-MalNEt into the 95 and 25k peptides, with little change in that of the 70k fragment. Thus, SH-C appears to be in the 25k fragment, which is analogous to the 22k fragment of skeletal myosin containing SH-1 and SH-2. ADP is more effective than ATP in accelerating the loss of K-ATPase, while Mg(II) or myosin phosphorylation have little effect. MalNEt increases the Ca(II) and Mg(II) ATPase activities, which involve blocking of either SH-A or SH-B. ATP accelerates the change, while the transition to the 10S form has the opposite effect. These results suggest that ATP induces one or more local conformational changes in myosin, which enhance the reactivity of SH-C and one of the rapidly blocked thiols. These changes do not involve the 6S-10S transition.

T-Pos228 PHOTOAFFINITY LABELING OF MYOSIN SUBFRAGMENT-1 BY 2-AZIDO-ADP, Jean C. Grammer[1], Joseph J. Czarnecki[2] and Ralph G. Yount[1], [1]Biochemistry/ Biophysics Program, Washington State Univ., Pullman, WA 99164 and [2]Institute Enzyme Research, Univ. of Wisconsin, Madison, WI 53706.

Subfragment-1 (SF-1) has been specifically labeled by a photoaffinity analog of ADP, 2-azido-ADP (2-N_3ADP). To decrease the concentration of the non-photolabile tetrazole form of 2-N_3ADP, the analog was equilibrated in 0.1 NHCl for 40 min at 22°C and neutralized just prior to use. Co(III) phenanthroline crosslinking of SH_1 and SH_2 [Wells and Yount, PNAS 76, 4966 (1979)] stably trapped 0.6-0.7 mols [^{3}H]2-N_3ADP per mol SF-1. Addition of increasing levels of ADP decreased the amount of [^{3}H]2-N_3ADP trapped, demonstrating the analog was trapped at the active site. SF-1 containing trapped [^{3}H]2-N_3ADP was purified to remove free nucleotide and irradiated through a pyrex filter by use of a 450 W Hg lamp. One-third of the trapped nucleotide was covalently incorporated. The amount of photoincorporation after 2 min irradiation was shown to be the same over a period of several hours after trapping, indicating that the open ring form of 2-N_3ADP was favored by the low polarity of the active site. Analysis of the photolabeled [^{3}H]2-N_3ADP·SF-1 by SDS-PAGE showed that > 99% of the radioactivity was located in the 95 kDa heavy chain fragment. Similar analyses of the same preparation after limited trypsin treatment showed that the label was essentially all in the 23 kDa N-terminal tryptic peptide. These results are in agreement with other photoaffinity studies [Okamoto and Yount, Biophys. J. 41, 298a (1983)] and give further evidence that the purine binding site of ATP involves amino acid residues near the N-terminal region of the heavy chain. Supported by grants from MDA and NIH (AM-05195).

T-Pos229 Rate of Trapping of ADP on S-1 Increases with Decreasing Temperature. Kathy Cunningham and John W. Shriver; Department of Medical Biochemistry, School of Medicine; and Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901. We have measured the rate of trapping of ADP on myosin S-1 from rabbit skeletal muscle. Chymotryptic S-1 (22 μM) was reacted with freshly sublimated para-phenylenedimaleimide (22 μM) in the presence of 18 μM ^{3}H-ADP in 50 mM TRIS (pH 8.0), 0.1 M KCl, and 5 mM $MgCl_2$. Aliquots were removed and the trapping reaction quenched with 0.02 M DTE and 0.01 M EDTA at specified time intervals. The S-1 was precipitated twice by $(NH_4)_2SO_4$, and the amount of trapped ADP was determined by scintillation counting. All data was obtained within three days of DEAE cellulose column chromatography of the S-1. The maximum amount of trapped ADP depended significantly on the age of the S-1 preparation, however the variation of the rate of trapping did not vary with the age of the protein. The maximum levels of trapped ADP were reached within five minutes of reaction initiation at 20°C. Upon decreasing the temperature to 4°C, the maximum level was reached in 1.5 minutes. The data may be fit with an apparent first-order rate constant of 0.02 s^{-1} at 20°C and 0.05 s^{-1} at 4°C. The variation of the rate of trapping with temperature cannot be readily explained with any model which assumes a single conformation of S-1·ADP. The simplest explanation is that the low temperature Mr*ADP state previously observed in ^{31}P NMR experiments (Shriver, J.W. and Sykes, B.D. Biochemistry 20, 2004 (1981) is more readily crosslinked than Mt*ADP, the 25°C state.

T-Pos230 REACTIVITIES OF THIOLS IN MYOSIN ROD: EFFECT OF MAGNESIUM AND IONIC STRENGTH. Barbara Pliszka & Renné Chen Lu. Dept. of Muscle Res., Boston Biomed. Res. Inst. and Dept. of Neuro., Harvard Med. Sch., Boston, MA 02114

There are six cysteines in each chain of myosin rod of rabbit skeletal muscle: three are in the S-2 portion, at residues 66, 108 and 410 (Lu & Lehrer, 1984). The other three are in the LMM portion, assigned at # 572, 600, and 770 on the basis of homology between the amino acid sequence in the vicinity of these thiols and that of the rod of nematode myosin (McLachlan & Karn, 1982). Since the thiols are distributed in different regions of the rod, measuring their reactivities under various conditions may provide information on the conformations of these regions. Myosin rod was carboxymethylated with radioactive iodoacetic acid under various conditions. The cysteine- containing peptides were isolated using HPLC from the tryptic digests, and the radioactivity incorporated into each thiol was measured. In the denatured state all six thiols were equally reactive. In the native state, all thiols have low reactivity, the reactivity of Cys-108 or 410 is only 0.1% of that in the denatured state, Cys-600 exhibited the highest reactivity, about 20 times that of Cys-410; Cys-66, 572, and 770 had 2-4 times that of Cys-410. When the rods formed filaments, the reactivities of all cysteines further decreased: Cys-66, 108 and 770 were reduced to 50%, while Cys-410, 572, and 600, located in the middle of the rod, were reduced to 20-30% of their reactivities in the monomeric form. In the presence of Mg^{2+} the reactivity of Cys-108 increased 20% whereas Cys-572 decreased 50%. The results are consistent with the view that metal ions affect the conformation of the rod. This may play a role in the mechanism of filament formation and the movement of crossbridges. (Supported by NIH and AHA.)

T-Pos231 PHOSPHORYLATION OF SKELETAL MUSCLE MYOSIN: EVIDENCE FOR SUBSTRATE INHIBITION. S.M. Pemrick and E.E. Harper, (Intro. by E.B. McGowan)Dept. of Biochemistry, SUNY, Downstate Medical Center, Brooklyn, NY 11203.

When time course profiles of phosphorylation were compared at low, intermediate and high concentrations (1,6,& 12 mg/ml) of soluble (S) myosin over a wide range of myosin LC kinase concentrations, an apparent substrate inhibition was observed in both the initial rate and the maximal level of percent L2P, the latter decreasing from more than 90% (1.0 mg/ml) to less than 50% (12.0 mg/ml). Below 1.0 mg/ml. no substrate inhibition was apparent and the reaction followed pseudo-first-order kinetics, in agreement with the report by Persechini and Stull (1984, Biochemistry 23, 4144). Substrate inhibition was specific: a) no Ca2+-insensitive kinase activity or phosphatase activity was present; b) the kinase remained active throughout the experiment; c) the effect was quantitatively reproducible over many protein preparations; d) inhibition could be rapidly reversed by diluting the myosin solution, but not by adding a large excess of LC kinase. With respect to the ability to attain 100% L2P the following order prevailed: dilute solutions were easier to phosphorylate in the filamentous (F) than in the S state, the latter being equivalent to intermediate concentrations of F myosin and the most difficult to phosphorylate were high concentrations of S myosin. These results negated non-specific myosin aggregation and ionic strength as inhibitory mechanisms, and indicated myosin-myosin interactions inhibit phosphorylation. (Sup.by NIH grant HL22401)

T-Pos232 SKELETAL MYOSIN FILAMENT ASSEMBLY: EFFECT OF MAGNESIUM ATP. Prokash K. Chowrashi and Frank A. Pepe, Dept. of Anatomy, Univ. of Pennsylvania, Philadelphia, PA 19104.

Although native filaments separated from skeletal muscle have a precisely determined length of about 1.5 μm (Morimoto and Harrington, J. Mol. Biol. 77:165-175, 1973), myosin filaments assembled from myosin solutions have generally shown a wide distribution in lengths (Huxley, J. Mol. Biol. 7: 281-308, 1963). Recently Pepe et al. (Biophys. J. 45:150a, 1984) have described a procedure for diluting the KCl concentration of myosin solutions which produces filaments with a length distribution comparable to that observed for separated native filaments. Additional studies have shown that the length distribution does not change significantly over a 24 hr period. Furthermore, if filaments are formed under two different conditions which give significantly different lengths and the two filament suspensions are mixed, the length distribution of each population in the mixture also does not change significantly over a 24 hr period. Having formed filaments with a precise length distribution around 1.5 μm, the addition of 1-5 mM $MgCl_2$ or the addition of 1-5 mM ATP leads to a reduction in the length of the filaments over a 24 hr period. This effect is strongest with the ATP where at concentrations of 5 mM the filaments are about 0.8-0.9 μm in length. Addition of Magnesium ATP has a different effect on the length distribution. At 1-5 mM the length distribution is significantly sharpened around 1.5 μm. If filaments are formed in the presence of 1 mM Magnesium ATP according to the standard procedure (Pepe et al., Biophys. J. 45:150a, 1984) the length distribution is very broad, but it becomes very sharp after about 6 hrs and does not change from 6-24 hrs. The addition of 1 mM Magnesium ATP to a mixture of two populations of filaments also results in a single sharp distribution around 1.5 μm over a 24 hr period. We conclude from these observations that Magnesium ATP is involved in some way in the precise assembly of the myosin filament.

T-Pos233 EFFECTS OF CARNOSINE ON MYOSIN ATPase. A POSSIBLE ROLE FOR LC2 IN Ca^{2+}-ACTIVATION. Koshy George and Paul Dreizen. Biophysics Graduate Program, SUNY Downstate Medical Center, Brooklyn, New York 11203.

The dipeptides carnosine (β-alanyl histidine) and anserine (β-alanyl methyl histidine) are present in skeletal muscle of most vertebrates, with concentrations as high as 20-30 mM in fast skeletal muscle. These dipeptides are major buffers in muscle, but their functional role has been ambiguous, except for a report (Avena and Bowen, 1968) that carnosine and anserine may activate myosin ATPase. We have further examined the effects of carnosine on steady-state ATPase of rabbit fast muscle myosin and its proteolytic fragments. At pH 7.0 and below, carnosine activates Ca-ATPaseof myosin (about 2-fold activation in 50 mM carnosine). At pH 8.0, there is slight decrease in specific activity, presumably related to increase in ionic strength. Identical results are obtained for column purified myosin and crude preparations of myosin. Essentially the same findings are also obtained for heavy meromyosin (HMM), although specific activity is augmented somewhat greater, ie., 3-fold in 50 mM carnosine. In contrast, in S-1 prepared by chymotryptic digestion of fast muscle myosin, carnosine results in decreased Ca-ATPase over the entire range from pH 6.4 to pH 8.0. Moreover, samples of HMM that are deficient in LC2 or have degraded LC2 show loss of carnosine activation. The overall evidence suggests that carnosine augments Ca^{2+}-activation of myosin, especially under conditions in which histidyl residues are protonated, and this effect would appear to involve some kind of interaction with LC2.

T-Pos234 REGULATED ACTIN INTERACTION WITH HMM AT PHYSIOLOGICAL IONIC STRENGTH IN THE PRESENCE OF ATP. Saleh C. El-Saleh and James D. Potter, Dept. of Pharmacology, Univ. of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

Over the past several years numerous conflicting reports have been made regarding the affinity of S-1 and HMM for regulated actin and whether or not this interaction is Ca^{2+} dependent. Since all of the previous studies were carried out at low ionic strength, questions have been raised as to their physiological relevance. Recently we have perfected a technique to measure the binding of HMM (85% intact LC-2) to actin at physiological ionic strength (μ = 134mM), using an ATP regenerating system (at 23-24°C). Our results were similar to those of Chalovich and Eisenberg (1984, Biophys. J. 45, 221a) at low ionic strength (19mM). The HMM affinity constants in the presence and absence of Ca^{2+} (although weaker than those at 19mM) were nearly identical [2.73 x $10^3 M^{-1}$ ($+Ca^{2+}$) and 2.54 x 10^3 M^{-1} ($-Ca^{2+}$)]. These results are also consistent with their conclusions, that, tropomyosin-troponin may not act by simply blocking the binding of cross-bridges to actin, but may inhibit a kinetic step in the ATPase cycle. One must be cautious in this interpretation, however, since HMM, although more intact than S-1, may not be equivalent to intact myosin assembled into the thick filament. Our results also show at least a 4 fold drop in the binding constant of HMM as compared to those at 19mM. This suggests that raising the ionic strength may cause a decrease in the number of cross-bridges attached under relaxing conditions which in turn may explain the very low stiffness of muscle fibers at μ = 170 mM (Brenner et al. 1983, P.N.A.S. 79, 7288). Supported by NIH HL226193A and the Muscular Dystrophy Association.

T-Pos235 BINDING OF PPi AND AMP-PNP TO CROSS-LINKED AND NON-CROSS-LINKED ACTO·S-1 by José A. Biosca, Lois E. Greene and Evan Eisenberg, NHLBI, NIH, Bethesda, MD 20205

We previously determined the binding constants of ADP, AMP-PNP, and PPi to acto·S-1 by measuring the dissociation of acto·S1 as a function of ATP analog concentration (Greene and Eisenberg, J. Biol. Chem. 255, 543 (1980)). In the present study, we reinvestigated this question by measuring the extent to which these ATP analogs inhibit the acto·S-1 ATPase activity using both cross-linked actin·S-1 and non cross-linked proteins; no significant difference was found between the cross-linked and non-cross-linked acto·S-1 complex in their affinity for either ADP or AMP-PNP. The binding constant of ADP to acto·S-1 determined by the kinetic method was in excellent agreement with that obtained previously by the dissociation method, both techniques giving values of $\sim$ 7 x 10^3 M^{-1}. However, this was not the case for both AMP-PNP and PPi, with the kinetic method giving $\sim$10-fold weaker binding constant than that determined by the dissociation method. Upon redoing our dissociation experiments over a wide range of actin concentrations, we find that the dissociation method gave too strong a value for these binding constants and there is now good agreement between the methods. The binding constants of PPi and AMP-PNP to acto·S-1 at μ = 70 mM, 25°C, are on the order of 5 x 10^2 M^{-1}; the extremely weak binding of these ATP analogs to acto·S-1 makes it difficult to obtain this value with great accuracy. The weak binding of AMP-PNP and PPi to acto·S-1 is consistent with the recent fiber studies of Schoenberg and Eisenberg (Biophys. Abst. 1985) who found that the rate of force decay after a stretch depended markedly on the concentration of either AMP-PNP or PPi.

T-Pos236 ATPase ACTIVITY OF REGULATED CROSS-LINKED ACTIN-S-1 by Lois Greene and Robert Thomas King, NHLBI, NIH, Bethesda, MD 20205 (Intr. by R. Adelstein, LMC, NHLBI, NIH, Bethesda, MD 20205)

Chalovich and Eisenberg (J. Biol. Chem. 257, 2432 (1982)) have suggested that at low ionic strength, troponin-tropomyosin regulates the actomyosin ATPase by inhibiting a kinetic step in the actomyosin ATPase cycle rather than by blocking the binding of S-1 to actin. This leads to the prediction that troponin-tropomyosin should inhibit the ATPase activity of acto·S-1 even when cross-linked to actin. In the present study, this was tested by using cross-linked actin-S-1 prepared according to Mornet et al. (Nature 292, 301). Troponin-tropomyosin inhibits the acto·S-1 ATPase activity of non-cross-linked proteins about 95%, less than the 99% expected based on low energy utilization in relaxed muscle, but still quite a significant degree of inhibition. In contrast with cross-linked actin-S-1, troponin-tropomyosin caused only 70% inhibition of the ATPase activity using a preparation containing high ratios of cross-linked S-1 to actin. However, this was increased to 90% if we used a preparation containing low S-1 to actin ratios which was made under mild crosslinking conditions. At μ=18 mM, 25°C, the ATPase activity of the latter cross-linked preparation was only about 2.5 fold greater than the maximal actin-activated ATPase of S-1 obtained with regulated actin in the absence of Ca^{2+}. Furthermore, at high ionic strength, troponin-tropomyosin caused about 95% inhibition of the ATPase activity of the cross-linked S-1 preparation. Since cross-linked S-1 appears to behave like S-1 in the presence of infinite actin concentration, inhibition of the ATPase is not due to blocking of the binding of S-1 to actin. Therefore, both at low and high ionic strength, these results are in agreement with the suggestion that troponin-tropomyosin regulates primarily by inhibiting a kinetic step in the ATPase cycle.

T-Pos237 THE DISSOCIATION OF REGULATORY LIGHT-CHAINS FROM SCALLOP MYOFIBRILS BY EDTA AT TEMPERATURES GREATER THAN 20° OCCURS BY A RANDOM PROCESS.

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There is a biphasic relationship between the actin-activated ATPase of scallop (Aequipecten irradians) myofibrils, in the absence of calcium, and the regulatory light-chain/myosin ratio, obtained when regulatory light-chains are readded to 35°-desensitized scallop myofibrils in the presence of magnesium (Chantler & Szent-Györgyi, 1980. J.Mol.Biol. 138. 473-492.). However, it is shown here that analagous ATPase curves, obtained from myofibrils by direct desensitization (10mM EDTA at various temperatures), are parabolic. One possible explanation is that the two heads of scallop myosin are equivalent, with respect to light-chain dissociation, in the absence of Mg^{++}. It is known, however, that regulatory light-chain dissociation from Aequipecten myofibrils, as a consequence of repeated EDTA washes at 0°, is biphasic (Kendrick-Jones et al., 1976. J.Mol.Biol.104. 747-775). This apparent contradiction is shown to be due to a temperature-dependent switch, operating in Aequipecten myofibrils between 17° and 23°. Below 17°, regulatory light-chains dissociate in a biphasic manner in the presence of EDTA; at 23° or above, regulatory light-chains dissociate in a random manner. For points obtained by direct dissociation at 17° or below, the theoretical curves relating actin-activated ATPase (-Ca++) to light-chain stoichiometry, for both biphasic and random dissociation mechanisms, are so close together that it is impossible to distinguish between the two models in this type of experiment below 17°, hence the parabolic appearance of the stoichiometry curve in the direct desensitization experiments. Author is an Established Investigator of the A.H.A. and supported by Grant No. AM 32858 (N.I.H.).

T-Pos238 BINDING SITE OF LC2 IN CARDIAC MYOSIN AND ITS INFLUENCE ON ENZYMATIC ACTIVITY.

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Removal of LC2 from cardiac myosin has been achieved by digestion with a neutral protease from myopathic hamsters. The LC2 cleavage resulted in an increase in Vmax of actin-activated ATPase (Bhan, *et al.*, 1981). Using chromatographed cardiac myosin, we have obtained an LC2-deficient myosin in which the Vmax for actin-activated ATPase decreased in a manner similar to skeletal muscle myosin. The response of cardiac myosin to LC2 removal by the protease was reinvestigated to clarify the discrepancy between the two results. In recent studies the myosin was routinely purified by ion-exchange chromatography, both before and after protease treatment. This procedure separates C-protein, the presence of which could have resulted in the originally reported increase in ATPase, in a manner described elsewhere (Yamamoto & Moos, 1983). Thus, when control, LC2-deficient and LC2-recombined myosins were assayed in the presence of equimolar amounts of dog heart C-protein, there was a 30% increase in Vmax of control and recombined myosins with no change in Vmax of LC2-deficient myosin. These results would implicate C-protein in a cooperative effect together with LC2 in the activation of cardiac myosin ATPase. In parallel experiments, electron microscopy revealed rounder S1 heads and a thinning of mass at the S1/S2 junction upon LC2 removal. In control and recombined myosins, S1 heads were "pear" shaped with noticeable mass in the "neck" region. Thus, the S1/S2 junction in cardiac myosin, too, appears to be the LC2-binding site as it is in scallop and skeletal muscle myosins. (Supported by NIH grants HL 26569 to SSM and GM 14237 to HSS).

T-Pos239 TRANSIENT KINETIC ANALYSIS OF ATP HYDROLYSIS BY CARDIAC MYOSIN SUBFRAGMENT 1. J. H. Hazzard and M. A. Cusanovich, Dept. of Biochemistry, University of Arizona, Tucson, AZ 85721.

A systematic analysis of the effects of pH, temperature and ionic strength on the rate constants for ATP binding (K_1k_{23}) and hydrolysis ($k_{34}+k_{43}$) by cardiac myosin has been carried out using a kinetically homogeneous preparation of bovine ventricular subfragment 1. The rate constants are determined from analysis of transient kinetics observed by fluorescence stopped-flow. Arrhenius plots for K_1k_{23} and $k_{34}+k_{43}$ yield values for E_a of 137 and 64 kJ/mol, respectively. From the effect of ionic strength on K_1k_{23} an active site charge of +2 was calculated using the parallel plate model for electrostatic interactions (Watkins,A.,Ph.D. Thesis,Univ. of AZ,1984). The pH-profile was determined over a range of 5.5 to 8.0 and exhibits an n=1 transition as expected for the ionization of a single group with a pK of 7.1 for both binding and hydrolysis, indicating the presence of histidine in the active site. Under all conditions studied, the calculated percent fluorescence enhancement is observed to decrease not only at high [ATP] but also at very low [ATP]. The change in the calculated enhancement with [ATP] decreases with decreasing temperature but is insensitive to changes in pH and ionic strength. Analysis of this data over the range of experimental conditions now available provides a thorough test of the proposed kinetic mechanism for binding and hydrolysis. Explicit solutions to rate expressions based on a consecutive 3-step mechanism as well as a simplified 2-step version permit the assignment of values to individual rate constants as well as to the fractional contributions of the two fluorescent intermediates. The 3-step mechanism is shown to fit data for the dependence of the observed rate constant and the relative fluorescence enhancement on [ATP] under most, but not all, conditions studied. Supported by NIH Grant HL28906.

T-Pos240 KINETIC RATES OF TRYPTIC DIGESTION OF BOVINE CARDIAC MYOFIBRILS. A. Azarcon*, D. Applegate, E. Reisler, Dept. of Chem. and Biochem. and the M.B.I., UCLA, L.A., CA. 90024

The interaction of cardiac myosin crossbridges with actin in the presence of nucleotides and pyrophosphate was probed by measuring the initial rates of cleavage of the 50K/20K junction of myosin heads in cardiac myofibrils. At 24^{0}C, physiological ionic strength and pH, this cleavage is slow but nonnegligible under rigor conditions and proceeds at a rapid rate in the presence of MgATP/1mM EGTA. Using the rates obtained in rigor and in the presence of MgATP as references, a reliable measure of the dissociation of crossbridges in cardiac myofibrils under various solvent conditions can be derived from the initial rates of cleavage of the myosin head. With this approach, we find that at 24^{0}C, under physiological salt conditions, MgADP, MgPP$_i$, and MgAMPPNP dissociate only a small fraction of cardiac crossbridges (10%, 5%, and 15%, respectively). These fractions of dissociated myosin heads are significantly increased at 4^{0}C. An underlying assumption in the foregoing is that in physiological salt in the presence of MgATP, crossbridges remain dissociated from actin during the tryptic cleavage of myofibrils. This is supported by the similarity of the proteolytic reaction in myofibrils under these conditions and in a "synthetic" relaxed state induced by 50% ethylene glycol in the presence of MgAMPPNP (Marston & Tregear, 1980; J.Mol.Biol. 139). When the solvent system contains 50% ethylene glycol, digestion of myosin is equally rapid in the presence of MgATP and MgAMPPNP; the rate in the presence of MgADP is unchanged from that of rigor. We also present evidence that in low salt medium (0.01M NaCl) a substantial fraction of myosin heads (30%) remain attached to actin in the presence of MgATP.
Supported by Laubisch Fund, an AHA postdoctoral fellowship and grants from USPHS(22031) and MDA.

T-Pos241 TISSUE SPECIFICITY OF cAMP-PHOSPHODIESTERASE INHIBITORS. D. Pang, W. Ingebretsen, A. Hagedorn, P. Erhardt, E. Ho, J. Wiggins. Berlex Laboratories, Inc., Cedar Knolls, NJ 07927

Among cAMP-phosphodiesterase (PDE) inhibitors, some (e.g., milrinone) are effective cardiotonics with no CNS actions, whereas others (e.g., rolipram) are effective antidepressants with no cardiotonic activity. To determine the nature of this specificity, we compared the inhibitory potencies of several cardiotonics (piroximone, fenoximone, milrinone, and CI-914) to 3-isobutyl-1-methylxanthine [IBMX] and their octanol/buffer partition coefficients. cAMP-phosphodiesterase activity was measured with radiolabelled cAMP and thin layer chromatography. In addition to these agents, we also examined more hydrophilic, close structural analogs of CI-914 and of rolipram (CK-2438 and ZK-73433, respectively).

	Fenox.	Pirox.	Milrin.	Amrin.	CI-914	CK-2438	Rolip	ZK	IBMX
Brain IC50	NR	NR	100	NR	NR	NR	0.8	50	33
Heart IC50	4	31	6	56	8	50	NR	NR	22
Part'n Coef	36	1.1	1.1	3.4	11	1.4	100	3.8	15

NR=Not reached at 100 uM; n=4 to 6

The cardiotonic agents are specific for enzyme isolated from heart, whereas rolipram is specific for the brain enzyme. There is no correlation between tissue specificity and lipophilicity, even among pairs of similar structures.

T-Pos242 PHARMACOLOGICAL MODULATION OF ARTERIAL MYOSIN PHOSPHORYLATION AND CARDIAC MYOFIBRILLAR ATPASE ACTIVITY. P. J. Silver, J.M. Ambrose, J. Dachiw, and P. B. Pinto, Division of Experimental Therapeutics, Wyeth Labs., Inc., Philadelphia, PA 19101

Regulation of contractile protein interactions in cardiac and vascular smooth muscle involves, respectively, Ca^{2+} binding to troponin C and calmodulin. We have quantitated the effects of known calmodulin antagonists, the Ca^{2+} blockers cinnarizine and perhexiline, and the cardiotonics Vardax and APP-201-533, on Ca^{2+}-regulated myosin light chain phosphorylation (MLCP) and actin-myosin interactions in native vascular actomyosin and cardiac myofibrils. Concomitant inhibition of arterial superprecipitation and MLCP by perhexiline (IC_{50}=33 μM) and cinnarizine (IC_{50}=60 μM) was similar to the calmodulin antagonist W-7 (IC_{50}=35 μM), and was characterized by a rightward shift in the pCa relationship, decreased maximum activity, and attenuation by exogenous calmodulin. Vardax and APP-201-533 both stimulated cardiac myofibrillar ATPase activity and had no effect on vascular MLCP. Various calmodulin antagonists, which all inhibited arterial MLCP, either stimulated (trifluoperazine, perhexiline, chlorpromazine, calmidazolium), had no significant effect on (pimozide, cinnarizine, compound 48/80), or inhibited (W-7, haloperidol) cardiac myofibrillar ATPase activity. All effects in myofibrils were independent of changes in MLCP. Perhexiline was more potent and efficacious than APP-201-533 and produced a leftward shift in the pCa relationship for myofibrillar ATPase activity. In summary, calmodulin antagonists differentially affect cardiac myofibrillar ATPase activity. It is possible with agents such as perhexiline and certain calmodulin antagonists to directly modulate Ca^{2+} sensitivity of both arterial (decrease) and cardiac (increase) contractile protein interactions.

T-Pos243 PURIFIED POPULATIONS OF DIFFERENTIATED MAMMALIAN CARDIAC MYOCYTES IN LONG-TERM CULTURE WITH SERUM-FREE CHEMICALLY-DEFINED MEDIA. Michael D. Schneider. Department of Medicine. Baylor College of Medicine. Houston, TX 77030.

In order to establish purified populations of mammalian cardiac ventricular myocytes in long-term culture, the effects of reduced fetal bovine serum (FBS) concentration or serum-free media were investigated, monitoring expression of creatine kinase (CK) isoenzymes. Primary cultures of Sprague-Dawley rat ventricle (0.1% trypsin:0.1% collagenase:0.025% DNase I) were inoculated at 5 x 10^4 cells/cm² in DMEM:Ham's F12 (1:1), 2 mM L-glutamine, 17 mM HEPES, 3 mM $NaHCO_3$, with 10% FBS (DF10) and at 30 h were fed DF10, medium with 0.5% FBS (DF0.5), or with 5 μg/ml insulin, 5 μg/ml transferrin, 10 μM hydrocortisone, 1 ng/ml angiotensin II, 1 nM Na_2SeO_4, and 1 nM LiCl (M19). By 4-6 d cultures in DF10 were confluent with fibroblasts, while spontaneously contracting myocytes comprised >95% of cells in DF0.5 or M19. Results for embryonic 21-d ventricle cultured 7 d (μg protein/cm², IU CK/g cell protein, and % MM:MB:BB) were: DF10: 4.35, 323, 45.4: 27.5:21.6; DF0.5: 2.01, 1295, 45.2:36.6:17.2; M19: 2.11, 1850, 49.4:34.2:16.4. Values after 14 d were: DF10: 4.18, 868, 24.7:18.6:47.6 (17.4% myocytes); DF0.5: 1.20, 644, 50.8:19:22.8 (50.5% myocytes); M19: 1.22, 2641, 50.2:28:11.6 (96.3% myocytes), with similar results after 22 d. Results for embryonic homogenates and for 14 d ventricle in vitro (M19) were (% MM:MB:BB): 14-d: 29.1:33.5:37.4; 21-d: 45.2:36.1:17; 14-d + 1d in vitro: 33.9:33:33.1; 14-d + 7d: 50.4:27.4:20.6. Thus, a serum-free chemically-defined medium, M19, can support (1) in vitro differentiation of early embryonic mammalian cardiac myocytes and (2) long-term culture of late embryonic cardiac myocytes which remain free of fibroblasts and retain their differentiated state.

T-Pos244 STRUCTURAL STUDIES OF EMBRYONIC CARDIAC MYOSINS. Robert R. Kulikowski. Department of Anatomy, Mount Sinai School of Medicine of the City University of New York, New York 10029.

Analyses of myosin isoforms present in the early developing heart have been based mostly on immunological criteria, using both poly- and monoclonal antibodies, and have yielded equivocal results as to structural differences among these isozymes. Studies presented here attempt to clarify this situation by directly examining the populations of myosin heavy chain (MHC) at very early stages of heart development using biochemical means. MHC were electrophoretically purified from the entire tubular hearts of chick embryos at 2.5 d of incubation and from the ventricles of embryos at 5.5 d of incubation. Electrophoretically homogeneous preparations were radiolabelled by using ^{125}I-Bolton-Hunter reagent followed by chemical cleavage with cyanogen bromide (CnBr) or proteolytic cleavage with V8 protease from S. aureus. The resultant peptides were separated by PAGE and visualized by radioautography. Single dimension peptide maps demonstrated a considerable homology in the pattern of fragments produced by CnBr, especially in the size range below 60 kD. However, several peptides in the range of 65-90 kD were prominent in the 5.5 d ventricular MHC but were not detectable in the 2.5 d preparations. V8 protease single dimension maps demonstrated a series of peptides in the 25-50 kD range in MHC from 2.5 d hearts, which were not found in 5.5 d hearts. The patterns of peptides below 25 kD exhibited a considerable homology, but were not identical. These results demonstrate that differences exist between the MHC populations of tubular hearts from 2.5 d embryos and those of ventricles at 5.5 d of incubation, and indicate that the ventricular isoform may not be a primordial type of myosin found throughout development. (Supported by Grant HL 30517 from the NIH.)

T-Pos245 ANOMALOUS LIGHT CHAINS IN HUMAN CARDIOMYOPATHY. K. Mabuchi, L. Papp*, Y. Mabuchi*, P. Sotonyi*, P. Allen, J. Gergely, and F.A. Sreter, Dept. of Anesth., Mass Gen. Hosp. and Harvar Med. Sch., Dept. of Muscle Res., Boston Biomed. Res. Inst., Dept. of Forensic Med., Medical Sch., Budapest and Dept. of Anesth., Brigham & Women's Hosp., Boston, MA 02114.

The presence of ventricular type light chains (VLC) in atria and atrial type light chains in ventricles of hypertrophic human hearts (Cummins, Biochem. J. 205, 195, 1982; Tuchschmid et al., in Cardiac Adaptation, Steinkopff 1982, p. 123; Sreter et al., ibid, p. 129) has been reported. In this work myosin extracted from human biopsy samples (1 to 10 mg, wet weight) of cardiomyopathic (CM) hearts, dilated or hypertrophic but without ischaemic disease, or from the removed hearts of recipients of heart transplants, was studied. Myosin was run on non dissociating pyrophosphate containing gels and the region of the isozyme bands was cut out and re-electrophoresed on SDS slab gels. In CM ventricles the mobility of VLC1 was faster than that of normal VLC1. On the other hand the mobility of VLC2 of CM hearts was somewhat slower than that of normal VLC2. Polyclonal antibody raised against normal VLC1 reacted also with VLC1 found in CM ventricles indicating their homology. Myosin light chains of CM ventricle and those of human skeletal slow muscle co-migrate on two dimensional gel electrophoresis, which suggests their identity. Digestion with trypsin or trypsin+papain of the isolated CM VLC1 produced a pattern different from that of normal VLC1. Immunoblots of the CM LC digests showed distinct differences from the normal ones; there were no changes in the peptide pattern of CM ventricular myosin heavy chains. Atria of some CM hearts show the presence of both atrial and ventricular type heavy and light chains (Supported by grants: NIH: HL-5949, HL23967, HL27231, NSF, MDA).

T-Pos246 IMMUNOCHEMICAL STUDIES OF DEVELOPMENT OF SLOW SKELETAL MUSCLE OF CHICKEN. Yatian Zhang and S.A. Shafiq. Biophysics Graduate Program and Department of Neurology, SUNY Downstate Medical Center, Brooklyn, N.Y.(Intr. by Manfred Brust)

The development of two slow muscles of the chicken, namely, anterior latissimus dorsi (ALD) and adductor profundus (AP) was studied at several embryonic and post-hatching stages. Three monoclonal antibodies (McAbs), ALD-180, ALD-88 and ALD-47 produced against slow myosin of the chicken were used in conjunction with two previously reported antibodies, namely, ALD-58 (anti-slow myosin) and MF-14 (anti-fast myosin). The specificity of these antibodies for myosin heavy chains (MHC) was established by radioimmune assay, immunoautoradiography and immuno-fluorescence. The immunocytochemical reactions of muscle fibers at 5 day embryonic, 16 day embryonic and early and late post-hatching stages revealed distinctive staining patterns. It is proposed that differences of immunoreactivity of the muscle fibers at the four stages indicate isozyme transitions. In addition, the maturation of fibers in ALD muscle (unlike of those in the fast pectoralis muscle) was found to be non-synchronous. By electrophoresis in 5% acrylamide SDS-gels, two distinct MHC bands were seen in the AP muscle of 16 and 20 day embryos. The two MHC bands showed striking changes during embryonic and post-hatching stages, their reactivity with McAbs and peptide map characteristics will be presented.

T-Pos247 CONTROL OF MYOSIN ISOZYMES TRANSITIONS IN THE DEVELOPING RAT. A.M. Kelly, B. Gambke, and N.A. Rubinstein, Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, PA 19104.

In developing muscles of locomotion in rat hind limb the adult fast isozymes, FM_1-FM_3, appear between 10 and 15 days of age. They gradually replace the developmental set of isozymes, f1 to f4, and are the sole isozymes by 30 days. This switch is regulated by thyroxine (Gambke et al., FEBS Letts 156:335, 1983) levels which are undetectable at birth and reach peak serum concentrations at 15 days. In the present study we examined when this isozyme shift occurred in non-locomotor muscles. In the diaphragm, all developmental isozymes f1-f4 are present at 18 days gestation which is earlier than in limb muscles. At 20 days gestation a faint band with the mobility of adult FM_3 is seen. This has the light chain complement of $LC1_f$:$LC2_f$ with small proportions of $LC1_s$. By 10 days FM_1-FM_3 predominate. In the tongue FM_3 is evident at birth and in the sternomastoid at 5 days of age. In all of these muscles developmental isozymes are eliminated by 30 days. In the masseter the adult isozymes do not appear until 15 days, developmental isozymes remain prominent at 30 days and traces can be detected at 70 days. These results suggest the signal to switch to adult myosin isozymes is related to the development of function and is not simply regulated by serum levels of thyroxine. In the diaphragm of hypothyroid baby animals the isozyme switch is delayed, but proceeds so that FM_1-FM_3 predominate at 15 days. The developmental isozymes, however, remain even at 30 days. Thyroxine thus does not initiate the switch from developmental to adult isozymes in the diaphragm but appears necessary to accelerate the change so that it proceeds to completion.

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T-Pos248 CHARACTERIZATION OF DEVELOPMENTAL AND DISEASE-RELATED MYOSIN HEAVY CHAIN ISOZYMES BY HPLC. **Rushbrook, J.I., Weiss, C., Wadewitz, A.G. and Stracher, A., Department of Biochemistry, SUNY-Downstate Medical Center, 450 Clarkson Ave., Brooklyn, N.Y. 11203.**

We have identified an atypical heavy chain in the myosin of the affected fast white muscle fibers of genetically dystrophic chickens (1,2). The aberrant protein is clearly unrelated to the normal embryonic isoform (1,2). Bandman has recently presented evidence that it is, in fact, the later-appearing neonatal isoform.

In order to further characterize both developmental and disease-related variants we have developed HPLC procedures for the purification and study of myosin and various of its proteolytic fragments. Using a reverse-phase system separating the major tryptic fragments of S-1, we have identified 50kDa species characteristic of normal and neonatal myosins. HPLC peptide-mapping of thermolysin digests of these fragments indicates minimal sequence differences between adult and neonatal heavy chains.

By these procedures, the atypical dystrophic heavy chain is found to resemble strongly the neonatal isoform. Suprisingly, it constitutes as much as 40% of the heavy chain quotient in adult chickens. (Supported by 1 R01 NS 843801 to A.S.)

(1) Rushbrook, J.I, and Stracher, A. (1979) Proc. Natl Acad. Sci (USA) 76, 4331-4334. (2) Rushbrook, J.I., Yuan A. I and Stracher, A. (1981) Cell. Motil.1, 399-416. (3) Bandman (1984) Muscle and Nerve 7, 312-326.

T-Pos249 MYOSIN LIGHT CHAIN COMPOSITION OF HINDLIMB AND FORELIMB MUSCLES OF DYSTROPHIC MICE OF DIFFERENT AGES. A.F.R. Stewart and D.J.Parry (Dept. Physiology, Univ. Ottawa, Ottawa, Ont. Canada)

In the mouse the Anterior Tibialis (AT) and Extensor Digitorum Longus (EDL) muscles of the hindlimb normally consist exclusively of type II (fast-twitch) fibres. However, in dystrophic (C57 BL/6J dy^{2J}/dy^{2J}) mice these muscles show significant prolongation of the twitch (1,2). We have suggested (3) that this may be accounted for by synthesis of slow myosin isoforms as a result of spontaneous twitching of the hindlimb muscles caused by lumbar root amyelination. The forelimb Extensor Carpi Radialis (ECR) shows neither spontaneous twitching nor slow myosin synthesis and relatively little prolongation of the time to peak tension. Slow myosin heavy chains may be visualised by means of immunohistochemistry (3). We now report the myosin light chain (MLC) composition of hind- and forelimb muscles from dy^{2J} mice of different ages. We have attempted to correlate changes in MLC composition with the pattern of activity recorded as spontaneous electromyographic activity in age-matched mice. Myosins were extracted as per Ref. 4. MLC patterns confirmed the immunohistochemical evidence of a shift in AT of older (>8 mo.) dy^{2J} mice towards slow myosin synthesis. Preliminary evidence suggests a greater increase in LC_{1s} than in LC_{2s} in some dy^{2J} AT. This would be consistent with the reported increase of the IM isoform of myosin in dy mice (5). Prolonged bursts of action potentials could be recorded from AT of mice aged >3 months, prior to this only single spikes or short bursts were seen. There appears to be a lag of at least 3 months before significant amounts of slow MLC are seen. No slow MLC were detected in ECR of dy^{2J} mice at any age. (Supported by Muscular Dystrophy Association of Canada).

T-Pos250 DANTROLENE REVERSES THE SYNDROME OF MALIGNANT HYPERTHERMIA BY REDUCING THE LEVEL OF INTRACELLULAR CA^{2+} CONCENTRATION. Lopez J.R., Alamo L.*, Jones D*, Allen P.D., Papp, L.*, Gergely J, and Sreter F.A., Centro de Biofisica y Bioquimica, IVIC, Caracas, Venezuela; Dept. of Anesth., Mass. Gen. Hosp.; Dept. of Muscle Res. Boston Biomed. Res. Inst.; and Dept. of Anesth., Brigham & Women's Hosp. Boston, MA 02114.

Administration of dantrolene sodium induces relaxation of malignant hyperthermia susceptible (MHS) fibers in which contracture has been elicited by triggering agents. We have investigated the effect of D on the intracellular $[Ca^{2+}]$ in porcine MHS skeletal musclefibers in vivo after the syndrome was triggered with halothane (H). The determination of free $[Ca^{2+}]$ was done with calcium selective electrodes as described previously (Lopez et al. Biophys. J. 43:1, 1983). The animals were anesthetized with a mixture of thiopental, N_2O, O_2, and fentanyl, and spontaneous movement was prevented with pancuronium. After baseline $[Ca^{2+}]$ measurements were made the syndrome was triggered with a 30 min. exposure to 3% H, and reversal of the pancuronium with neostigmine. When the syndrome was triggered the $[Ca^{2+}]$ rapidly increased from 0.47 ± 0.06 uM (mean $\pm$ SEM, n=27) to 10.62 ± 3.16 uM (n=8). With the electrodes in place, D, 1 mg/kg, was administered intravenously. Intracellular $[Ca^{2+}]$ abruptly decreased to 0.35 ± 0.01 uM (n=6) and the hypermetabolic state abated. These results show that MH is accompanied by an increase in intracellular free $[Ca^{2+}]$ and the effect of D is associated with a decrease. (Supported by grants: NIH GM-15904, HL27231, HL-5949; NSF, MDA, Conicit S1-1277, S1-1148)

T-Pos251 FREE MYOPLASMIC CALCIUM CONCENTRATION IN SKELETAL FIBERS FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE SWINE, MEASURED IN VIVO WITH CA^{2+} SELECTIVE MICRO-ELECTRODES. Lopez J.R., Alamo L.* Jones D.*, Allen P.D., Papp L.*, Gergely J., and Sreter F.A., (Intr. by S. Sarkar) Centro de Biofisica y Bioquimica, IVIC, Caracas Venezuela; Dept. Anesth., Mass. Gen. Hosp., Dept. of Muscle Res., Boston Biomed. Res. Inst., Dept. Anesth., Brigham and Women's Hosp., Boston MA 02114

Malignant hyperthermia (MH) is a genetic syndrome usually initiated by exposure to volatile anesthetic agents and/or pharmaco-depolarizing muscle relaxants. We have used a Ca^{2+} selective microelectrode prepared and calibrated as described previously (Lopez et al., Biophys J. 43:1, 1983) to measure in vivo the intracellular free Ca^{2+} concentration in skeletal muscle fibers of MH susceptible (Poland China) and control (Yorkshire) swine. The swine were anesthetized with thiopental, N_2O, O_2, and fentanyl. Spontaneous movement was prevented with pancuronium. The resting membrane potential and the calcium potential were measured in superficial fibers of the tibialis anterior muscle. In the susceptible animals $[Ca^{2+}]$ was 0.47 ± 0.06 μM (mean±SEM, n=27) while in controls it was 0.12 ± 0.01 μM (n=20). There was no significant difference in the resting membrane potential between susceptible fibers (-82±1.76 mV) and control fibers (-83.3±1.06 mV). These results support the hypothesis that higher levels of ionized calcium in the myoplasm are associated with susceptibility to MH. (Supported by:NIH Grants GM-15904, HL-5949, HL 27231, and by NSF, MDA, Conicit S1-1277, S1-1148).

T-Pos252 CHANGES IN IONIZED CALCIUM CONCENTRATION IN STIMULATED MUSCLE. F. A. Sreter, J.R. Lopez, L. Alamo*, L. Papp*, K. Mabuchi and J. Gergely, Dept. Muscle Res., Boston Biomed. Res. Inst.; Depts. of Neurology and Anesthesia, Mass. Gen. Hosp.; Dept. Biol. Chem., Harvard Med. School,Boston, MA.; and Centro de Biofisica y Bioquimica, IVIC, Caracas, Venezuela.

Electrical stimulation through the nerve leads to transformation of fast fibers (type 2) to slow ones (type 1), the change occurring in the sequence 2B → 2A → 1. Since increased muscle activity is associated with a transient increase in sarcoplasmic Ca^{2+} concentration it seemed of interest to look into changes in $[Ca^{2+}]$ the course of fast → slow transformation of fibers of chronically stimulated extensor digit. longus muscle. Individual fibers were impaled first with a potential- and then a Ca^{2+}-sensitive microelectrodes (Lopez et al., Biophys. J. 43, 1, 1983). During the first ten days of stimulation $[Ca^{2+}]$ increased from about 0.1 uM to 0.5 uM and declined in about 30 days to a value about twice the normal. By this time, in terms of the myosin isozyme pattern and histochemical metabolic enzyme profile the majority of the fibers had changed from fast to slow. The rise and decline of $[Ca^{2+}]$ was preceded by a transient increase in total calcium by a factor of ~4, as also reported previously (Sreter et al., in Plasticity of Muscle, ed. D. Pette, de Gruyter, Berlin, New York, p. 441, 1982). Unstimulated fast and slow (e.g. EDL and soleus) muscles do not differ in their $[Ca^{2+}]$ levels; thus it appears that the transformation process itself is accompanied, particularly in its earlier stages, by elevated $[Ca^{2+}]$ levels. The mechanism by which Ca^{2+} initiates the observed change in gene expression will require further work. (Supported by grants: NIH: HL-5949, HL23967, MDA; I.N.H., Conicit S1-1277,S1-1148).

T-Pos253 SEQUENCE EVIDENCE RELATING TO THE ORIGIN AND EARLY EVOLUTION OF LIFE.
David G. George, Lois T. Hunt, and Winona C. Barker. National Biomedical Research Fdn., Georgetown University Medical Center, Washington, D.C. 20007

Many proteins and nucleic acids can be considered to be ancient in that their sequences preserve a record of early evolutionary history. Of particular interest are the ferredoxins, cytochrome c-type proteins, and the ribosomal RNAs. These molecules are found in a wide variety of organisms spanning the prokaryotic and eukaryotic kingdoms. Their sequences provide the basis for a composite phylogenetic tree outlining the major Precambrian evolutionary events. Ferredoxin has long been known to contain an internal gene duplication that predates the divergence of all present-day organisms. This duplication represents a point of earliest time and provides a base for the phylogenetic tree. Recent evidence suggesting the occurrence of an internal gene transposition has required us to reassess aspects of our interpretation of ferredoxin evolution. Our current understanding of the evolution of the bacterial ferredoxins and implications for the interpretation of the composite phylogenetic tree will be discussed.

This work was partially supported by NASA contract NASW3954 and NIH grant RR01821.

T-Pos254 ON FINDING TOO MANY SEQUENCE HOMOLOGIES. Winona C. Barker and David G. George, National Biomedical Research Foundation, Washington D.C., 20007

Recently new tools have become more widely available for the analysis and comparison of protein sequence data. The Protein Sequence Database of the National Biomedical Research Foundation is available on magnetic tape, on-line, and on floppy diskettes through various suppliers, usually in conjunction with programs for comparison of a sequence to another or to the database. As the sequence data are rapidly accumulating, the searching algorithms are more efficient and more thorough, and more researchers are using these tools, the finding of unexpected similarities (often mistakenly called "homologies") is occurring more frequently. The significance of these similarities is very difficult to assess and statistical measures are often misused and misinterpreted. In 1972, when the protein sequence database was distributed as a deck of punched cards containing 518 sequences with 48,292 amino acids, we had developed guidelines to assist our staff in deciding if two sequences are related. About ten years later, when the database contained 1667 sequences with 258,126 residues, it became necessary to reevaluate our previously established criteria. With the increased amount of data, the expectation of finding sequences that achieve high similarity scores by chance alone had dramatically increased; thus it was necessary to adopt more stringent criteria. We find ourselves in a similar situation today. The general problem of inferring functional and evolutionary relatedness from similarity measures will be addressed and progress on the establishment of guidelines to assess the significance of sequence similarity will be presented.

This work is supported by NIH grants RR01821 and HD09547.

T-Pos255 ANALYSIS OF MYCOPLASMA 5S rRNA SEQUENCES: EVOLUTION OF THE SMALLEST CELLS.
Jack Maniloff*, M.J. Rogers+, J. Simmons+, R.T. Walker+, W.G. Weisburg#, and C.R. Woese#.
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Mycoplasmas have been of interest to biophysicists, since these microorganisms have the smallest genomes of any free-living cells and, hence, should be the simplest living systems. Some mycoplasma genera have genomes of about 1000 Mdaltons and others of about 500 Mdaltons. The origin and phylogeny of these cells reflect the operation of natural selection within the constraints of limited genome complexity. We have used 5S rRNA sequence analysis to examine mycoplasma phylogeny. This shows that mycoplasmas form a coherent phylogenetic group, which arose by degenerative evolution as a branch of the low G+C Gram-positive tree near the lactobacilli and streptococci. The initial event was formation of a branch containing the saprophytic mycoplasmas; hence, cell wall loss probably occurred at the time of genome reduction to about 1000 Mdaltons. A subsequent branching produced the sterol-requiring mycoplasmas. During development of this branch, several independent genome reductions occurred, each producing a branch of sterol-requiring mycoplasmas with genomes of about 500 Mdaltons. Mycoplasma 5S rRNAs are smaller than eubacterial 5S rRNAs, as a result of the accumulation of small independent deletions in helix E and its loop. Mycoplasmas, particularly those with the smallest genomes, appear to have high mutation rates. This has allowed them to explore areas of evolutionary phase space requiring multiple compensating mutations, and suggests that they are in a state of rapid evolution. (Supported by USPHS NIH grant GM32442)

T-Pos256 ANALYSIS AND THEORETICAL SOLUTION OF THE 'LENS PARADOX' OF THE HUMAN VISUAL SYSTEM
Jane F. Koretz, Dept. of Biology, Rensselaer Polytechnic Institute, Troy, NY 12181

The human eye is a binocular visual system, with the cornea providing a fixed refractive power and the crystalline lens a small variable refractive power through controlled changes in its thickness and shape. The lens' refractive contribution has historically been attributed to changes in surface curvatures; these, combined with the small difference in refractive index between the lens and its surrounding environment, are believed to account for changes of up to 15 diopters in the young visual system. In terms of this mechanism, the age-related loss of near-focus abilities (presbyopia) would then be due to a flattening of anterior and posterior lens surfaces, as little or no change in the average refractive index of the lens has been seen. In fact, the opposite - an increase in sharpness of lens curvatures with increasing age - is observed (e.g., Brown, 1973; Koretz et al., 1984), indicating that the lens' refractive power arises in more complex fashion. It is suggested that the zones of discontinuity, regions of variable scattering power that increase in both number and contrast between adjacent bands with age, act as a set of nested lenses to determine the overall refractive contribution of the lens. Simulations illustrating these points will be presented.

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T-Pos257 COMPARATIVE ANALYSIS OF RADIOLIGAND AND FLUORESCENT BINDING.
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The exact expression for the percent binding, B, of a radioactive substrate, S, to an enzyme containing two different binding sites is:

$$B = X[S] + 2Y[S]^2 / (1 + X[S] + Y[S]^2) \qquad (1)$$

where $X = K_1 + K_2$ and $Y = K_1 \cdot K_3$. The K's are the three independent binding constants ($K_4 = K_1K_3/K_2$) of this system and may or may not be equal. Eq. (1) shows that B is dependent solely on the values of X and Y. Thus, any three constants resulting in the same X and Y will yield the same B and hence the same Scatchard plot. A single set of data can therefore be interpreted as sites having negative cooperativity, positive cooperativity, or lacking cooperativity. This means that neither the degree of cooperativity nor the heterogeneity of the sites may be inferred from the curvature of a Scatchard plot or any other plot using the binding data.

The binding of substrate to an enzyme can also be measured by monitoring the change in the fluorescence intensity of a fluorescent probe attached to the enzyme molecule. Assuming an instantaneous response of the probe to the binding of the substrate, the % change of fluorescence intensity is:

$$F = Z[S] + Y[S]^2 / (1 + X[S] + Y[S]^2),$$

where Z is not necessarily equal to X. The different binding curves obtained for unequal values of X and Z are discussed.

T-Pos258 A NETWORK THERMODYNAMIC MODEL OF CELL VOLUME REGULATION.
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Network thermodynamic models provide a means of studying the physical consequences of organization in biological systems. This unique ability is inherent in the network thermodynamic paradigm as the informational content of the system's topology is automatically incorporated in the model. The importance of this information can be illustrated in the process of constructing a model of the phenomenon of cell volume regulation.

Cell volume regulation is a swelling-shrinking phenomenon associated with osmoic regulation in cells placed in a hypoosmotic environment. Lymphocytes, given a hypertonic shock swell to 1.5-2 x normal volume within 2 min. This is followed by a 15-20 min. shrinking phase back to the cell's normal volume. This is a complex process involving the coupled flows of H_2O, Na^+, K^+, and other cellular constituents that has recently received considerable attention. Several models have been used to explain the experimental observations; yet none of these translates into a completely satisfactory quantitative model. In the process of constructing a network thermodynamic model to simulate this phenomenon the importance of the compliance of the cell membrane and the cell's nonionic constituents becomes clear. These are generally not considered in qualitative models, yet the shrinking phase can not be explained without considering the cell's compliance, and the nonionic constituents are an integral part of cellular osmo-regulation. The advantages of network thermodynamic models is further illustrated by expanding the original cell model to include a nucleus as an initial step in studying the effects of compartmentalization on osmotic regulation in cells.

T-Pos259 THEORETICAL ANALYSIS OF THE RECRYSTALLIZATION ENTHALPY OF ICE IN WATER
Allen Hirsh (Intr. by Harold T. Meryman), American Red Cross Research Laboratories, Bethesda, Maryland

Intracellular ice formation is a major source of cell injury during freezing. It is not known whether this damage is primarily caused by recystallization during warming or, alternatively, the increase in intracellular solution concentration accompanying formation of ice during warming (devitrification). The formation of homogeneous nuclei in supercooled water solutions can be theoretically correlated to the surface enthalpy of the nuclei. Since cells do not appear to contain heterogeneous nuclei, the prevention of intracellular homogeneous nucleation is prerequisite to avoidance of intracellular freezing. This study was an effort to predict the calorimetric behavior of ice during recrystallization.

This analysis of the surface enthalpy of ice begins with a classic thermodynamic derivation of the surface enthalpy of a crystal in its melt. The derivation is more accurate than those previously published becausue it takes into account the dependence of the surface free energy, γ, on the mean radius of curvature, r, of the ice crystals. Thus the recystallization enthalpy depends on: (1) γ, (2) the initial and the final mean crystal radii, (3) the surface layer thickness, δ, and (4) the variation of γ with temperature, $\frac{\partial \gamma}{\partial T}$. It is shown that for many published values of $\frac{\partial \gamma}{\partial T}$, the predicted enthalpy of recrystallization is positive, an improbable result which casts doubt on those measurements. Nevertheless, is shown that some published values of γ and $\frac{\partial \gamma}{\partial T}$ are consistent with the large negative enthalpy of recrystallization of homogeneous nuclei predicted from nucleation theory.

T-Pos260 PURINE METABOLISM IN HUMAN CELLS: CONSTRUCTION OF A STEADY STATE MODEL TO PREDICT METABOLIC CONSEQUENCES OF INBORN ERRORS. Mary Jean C. Holland, Department of Natural Sciences, Baruch College of the City University of New York, New York, NY 10010, and Department of Psychiatry, New York University Medical Center, New York, NY 10016.

Overproduction of purines is the cause of about 25% of all cases of primary gout. Abnormally high rates of purine synthesis can be demonstrated in vitro in intact skin fibroblasts derived from these patients. Although inborn errors of metabolism are thought to be the cause of primary gout, defects leading to overproduction have been demomstrated in only two of the many enzymes involved in purine synthesis and those defects account for only a small percentage of cases of overproduction. In order to examine the possibility that small changes in other enzymes may also lead to purine overproduction, a mathematical model was constructed to describe purine metabolism in the cells of normal subjects. For each of eight key metabolites, steady state concentrations were estimated from equations expressing rate of change in metabolite concentration as a function of rates of synthesis and utilization. These latter rates were predicted from kinetic constants and specific activities of relevant enzymes. Estimates produced by the model were compared with pool sizes as measured in cultured skin fibroblasts obtained from normal subjects. Model parameters were adjusted as needed to provide agreement between calculated and experimentally determined values. The two enzymatic defects known to result in purine overproduction were introduced separately into the model and their effects on steady state pool sizes of purine intermediates and on the rate of purine synthesis de novo were examined. The effects of small changes in kinetic properties of nine other enzymes were similarly evaluated.

T-Pos261 ELECTROTONIC SIMULATIONS OF HIPPOCAMPAL PYRAMIDAL NEURONS USING LADDER. N.T. Carnevale, F.J. Lebeda, and D. Johnston (Intr. by G. Plishker). Depts. of Neurol. & Neurobiol., SUNY, Stony Brook, NY and Dept. of Neurol. & Neurosci. Prog., Baylor Coll. Med., Houston, TX.

The recent application of voltage-clamp techniques to mammalian cortical neurons has created the need for evaluating the effects of nonisopotentialities using compartmental modeling methods (Johnston & Brown, J. Neurophysiol. 50:464). We have developed software that enables us to simulate the spread of voltage, current, and charge in hippocampal neurons under current- or voltage-clamp conditions and that uses realistic time-varying conductance changes to represent either excitatory or inhibitory synaptic inputs. The neuron is modeled as as a ladder network comprised of resistive and capacitative elements, and the resulting set of simultaneous, linear difference equations are solved by implicit integration. The editor program, which facilitates the creation and revision of models, and the simulator program (LADDER), which generates solutions under user-specified conditions, were designed to be fast, compact, and convenient. LADDER has been used to investigate the overlapping excitatory and inhibitory conductance changes that occur following mossy fiber stimulation in hippocampal neurons (Brown & Johnston J. Neurophysiol. 50:487). Furthermore, we have investigated the synaptic charge distribution in voltage- and current-clamp modes, the effects of voltage-clamp error (inadequate gain) on the measured synaptic conductances, and, by using the amplitude and kinetics of the measured current and voltage waveforms, the predicted electrotonic distance of the synapses from the recording site. One particular advantage of the form of model chosen is its ability to be easily extended to incorporate arbitrary branching patterns and voltage-dependent conductances. (Supported by USAMRDC DAMD17-82-C-2254, NIH grants NS15772 and NS11535, and McKnight Found. Develop. Award.)

T-Pos262 ANALYSIS OF SOLVENT RELAXATION AND EXCITED STATE PROCESSES IN FLUORESCENCE DECAY EXPERIMENTS. Joseph M. Beechem(1), Marcel Ameloot(1), Robert P. DeToma(2) and Ludwig Brand(1). (1) The Johns Hopkins University, (2) Loyola College, Baltimore, Maryland 21218.

Simultaneous analysis of multiple fluorescence decay curves has been utilized to obtain accurate recovery of decay parameters and to discriminate between competitive models. By analyzing fluorescence decay curves obtained through the entire emission spectrum, lifetime associated spectra can be obtained. However, in an excited state reaction, these spectra do not correspond to the individually emitting states. We will describe a procedure which utilizes all the information contained in the total fluorescence decay surface to resolve the spectra of the differently emitting states by directly fitting for the rate constants of the excited state process. Excited state solvent relaxation processes can be analyzed in this way as a discrete compartmental system. Results of the application of these procedures to the relaxation of 2-anilinonaphthalene in cyclohexane doped with ethanol will be described.

T-Pos263 CIRCULARLY INTENSITY DIFFERENTIAL SCATTERING OF LARGE CHIRAL AGGREGATES, **K. Sam Wells, David Beach, David Keller, and Carlos Bustamante**

Circularly Intensity Differential Scattering (CIDS) can provide information about the structural organization and helical parameters of large chiral molecules and chiral aggregates. CIDS is the ability of chiral molecules to scatter different amounts of left and right circularly polarized light as a function of the scattering angle. Recent theoretical treatments of CIDS have stimulated the design and construction of a CIDS instrument in our laboratory.

We have recently obtained preliminary results on the CIDS of certain cholesteric and blue phase liquid crystals. Also, experiments are being conducted on the helical sperm head of the octupus Eledone cirrhosa as well as condensed forms of DNA. The CIDS is shown to be much more sensitive to helical parameters than total scattering and provides structural detail in a unique way. Experiments have shown that CIDS is indeed sensitive to the pitch, radius and handedness of the helices as predicted by the theory. A simple model for the cholesteric liquid crystal shows close agreement with experimental results.

T-Pos264 LIPID VESICLE ENCAPSULATION OF SULFORHODAMINE 101 FOR THE STUDY OF FLUORESCENCE SELF QUENCHING MECHANISMS. A.L.Plant, B.L.Justus and P.E.Schoen. Chemistry and Optical Sciences Divisions, U.S.Naval Research Laboratory,Code 6190, Washington, D.C. 20375.

Concentration dependent self quenching of fluorescein-like dyes is a well known and widely used phenomenon in biochemistry and immunology. Suggested mechanisms of this quenching include fluorescence reabsorption, static "dark" complex formation, energy transfer, and collisional quenching. Although fluorescence lifetime measurements may provide evidence for distinguishing between the above processes, accurate measurements are not readily performed on solutions in which self quenching occurs because of the large extinction coefficients of these molecules. For those concentrations at which self quenching occurs, errors in lifetime measurements may be caused by the inability to prevent reabsorption of fluorescence by ground state molecules. Sulforhodamine 101 has been encapsulated into single bilayer phospholipid vesicles of 2000Å diameter in order to allow measurement of lifetimes in solutions with high microscopic concentrations (up to 100mM) and low solution concentration (ca. 0.02 OD). As a result, accurate fluorescence lifetime measurements can be made without distortion due to reabsorption. Sulforhodamine fluorescence lifetimes were observed to decrease as a function of encapsulated concentration. At an encapsulated concentration of 4mM, fluorescence intensity was quenched by a factor of 7, and the apparent lifetime decreased from 2.8 to 0.7 nsec, demonstrating the importance of dynamic collisional quenching in this system. After lysing vesicles with detergent the fluorescence lifetimes returned to their unquenched values.

T-Pos265 TRANSIENT ELECTRIC BIREFRINGENCE OF DILUTE RIGID-BODY SUSPENSIONS AT LOW FIELD STRENGTHS.
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Past expressions for rigid-body birefringence dynamics have assumed (a) particles with cylindrical symmetry, and (b) electric dipoles which are permanent and/or instantaneously induced. Both restrictions must be overcome in order to interpret recent experiments on DNA, macromolecules with irregular shapes, and lipid membranes with embedded proteins undergoing surface diffusion. We report on progress towards this goal.

Transient expressions are derived in the Kerr limit for a dilute monodisperse suspension of arbitrarily-shaped rigid-bodies without any slowly induced dipoles. We consider rotational diffusion (i) in a steady field, (ii) following reversal of field direction, or (iii) in a sinusoidally varying field. Besides dipoles, net electric charge may have an influence for screwlike shapes that couple rotations and translations. Results are given for a long bent rod.

When slowly induced dipoles are present, the dipole is no longer a function of orientation, but an independent parameter whose present value reflects a body's past history. A distribution of induced components exists at any orientation. A master equation is presented to describe the evolution of the distribution of orientations and induced dipoles. Simulation techniques developed elsewhere for Brownian motion are shown to be easily applied to obtain representative ensemble behavior. Results are given in the Kerr limit for a long straight rod with a variety of induction time constants.

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T-Pos266 **LOADING SMALL CELLS WITH METALLOCHROMIC INDICATORS.** Erik Wiener, Bernadette Lyons-Giordano, George Dubyak, and Antonio Scarpa. Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104.

Measurements of intracellular free calcium concentrations, $[Ca^{++}]_i$, using metallochromic indicators are generally limited to large cells since the indicators' polarity and differential molar absorptivity require their microinjection at relatively large concentrations. We circumvent this problem by using the "osmotic lysis of pinosomes" technique developed for loading macromolecules into small cells (Okada and Rechsteiner, Cell 29:33-41, 1982). We have successfully loaded Ehrlich Ascites tumor cells with enough phenol red, arsenazo III, or antipyrylazo III to provide intracellular concentrations that yield measurable signals from the internalized dye. Using antipyrylazo III, we show that in metabolically active Ascites Tumor cells the calcium ionophore A23187 raises the $[Ca^{++}]_i$ only slightly. In contrast, metabolically inhibited Ascites cells show a large rise in the $[Ca^{++}]_i$ upon addition of the ionophore A23187. Metabolic activity was determined by measuring the adenine nucleotides after incubating the cells for 15 minutes in 10 mM glucose and separating the nucleotides by High Pressure Liquid Chromatography. Virtually identical ATP/ADP ratios were found in treated cells and controls. Although we have internalized workable concentrations of dye using this technique and have kinetically monitored changes in the intracellular calcium concentration, we must determine to what extent the internalized dye is free in solution in the cytosol or compartmentalized. Supported by NIH grants HL-18708 and HL-07502.

T-Pos267 A SECTOR RULES FOR THE 175 NM CIRCULAR DICHROISM BAND OF SACCHARIDES, E.S. Stevens, Department of Chemistry, SUNY, Binghamton, N.Y. 13901

Circular dichroism measurements of saccharides, polysaccharides including glycosaminoglycans, and glycoproteins have recently been extended to 170 nm in solution. In order to develop the interpretation of these data, a sector rule is proposed to account for the CD band observed near 175 nm in aqueous solutions. The rule is based on assignment of the 175 nm dichroism to transitions centered on the oxygen atoms of the acetal (or hemiacetal) group, i.e., the ring oxygen O(5) and the linkage oxygen O(1). The transitions centered on hydroxyl oxygen atoms O(2), O(3), O(4), and O(6) are taken to have secondary importance. Each acetal oxygen atom interacts with the other so as to break the symmetry of the C-O-C chromophore, thus establishing a simple planar rule. Otherwise the two chromophoric oxygen atoms act separately and additively. When the chromophoric C-O-C group is aligned vertically with the oxygen closest to the observer and such that the other of the two oxygen atoms is attached to the upper carbon atom, positive dichroism is induced by perturbing groups on the left hand side of the plane and negative dichroism is induced by groups on the right hand side, with oxygen atoms acting as the strongest perturbers. Application of the rule to methyl aldopyranosides accounts for the experimental data and identifies the two dominant sources of dichroism as (1) perturbation of the ring oxygen transition by the O(1) oxygen to give strong positive CD in the α-linkage but not the β-linkage; and (2) strong negative dichroism in the ring oxygen transition induced by an axial O(4) atom. The rule is also applied to polysaccharides.

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