T-AM-Pos1 EFFECTS OF EGTA ON AEQUORIN LUMINESCENCE. E.B. Ridgway and A.E. Snow Department of Physiology and Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.
EGTA (ethyleneglycol-bis-(β-aminoethyl ether)-N,N-tetraacetic acid) inhibits the light emitting reaction of aequorin. This inhibition occurs through at least two mechanisms: 1) an indirect effect due to the well known calcium chelating property of EGTA which reduces the calcium concentration; and 2) a direct inhibitory effect of EGTA on the aequorin molecule presumably by binding. Evidence for the direct effect of EGTA on the aequorin reaction comes from several experiments. First, the initial velocity of the aequorin reaction is slower at any fixed buffered calcium concentration in the presence of high (50mM) EGTA-total than low (50μM) EGTA-total. Second, when a series of EGTA-calcium buffers is made up at constant free-EGTA (rather than the usual constant EGTA-total) the slope of the reaction velocity versus pCa curve is reduced, implying that the inhibitory agent is free-EGTA, not the EGTA-calcium complex. Finally, measurements of the quantum yield of the aequorin reaction show a reduced yield in the presence of high (10mM) EGTA as compared to that in low (10μM) EGTA. These inhibitory effects of EGTA are similar to those described for EDTA by Shimomura and Shimomura (FEBS Letters 138: 201, 1982). Because EGTA has a direct inhibitory effect on the aequorin reaction, care should be taken when using Calcium-EGTA buffers to calibrate an aequorin based system. These findings suggest that EGTA may inhibit or affect other calcium sensitive proteins. Supported by NIH Grant NS-10919.

T-AM-Pos2 MEASUREMENTS OF INTRACELLULAR FREE Ca^{2+} IN SKELETAL MUSCLE USING Ca MICROELECTRODES. López, J.R., Alamo, L., Caputo, C., Vergara, J. and DiPolo, R. (Intr. by Fulgencio Proverbio) . Centro de Biofísica y Bioquímica, IVIC, Apdo. 1827, Caracas, Venezuela and Physiol.Dept.,UCLA, Los Angeles, CA, USA.
Intracellular free calcium concentration was determined in sartorius muscle fibers, (Rana pipiens) using Ca^{2+} selective microelectrodes containing neutral ligand sensor resin (kindly given by Prof. Simon). Glass microelectrodes with tip outer diameter of about 0.3 μm. were pulled from borosilicate glass capillaries. The microelectrodes were siliconized with tri-n-butylchlorosilane, and then back filled with the calcium sensor. The microelectrodes were calibrated before and after intracellular measurements using solutions with $[Ca^{2+}]$ ranging from pCa3 to pCa9. Only those electrodes that showed a Nerstian behavior between pCa3 and pCa7 (28.5 mV per decade) were used . In quiescent skeletal muscle fibers which had a resting membrane potential not lesser than -85 mV, the mean basal pCa was 6.91±0.09 (SE) n=60, which correspond to a mean free myoplasmic calcium concentration of 0.12 μM (21°C). Caffeine 1 or 2 mM did not change the basal level of free cytotalic Ca^{2+} but 3 mM caused increase of $[Ca^{2+}]_I$ to 0.31 μM. Raising the external $[K^+]$ increases the metabolic rate of skeletal muscle (Solandt effect) . Treatment of muscle fibers with solutions containing 4,8,12 and 16 mM K^+, depolarize the muscle membrane from -89 mV to -82, -73, -68, -63 mV and increases the free calcium concentration to 0.15, 0.29, 0.47 and 0.56 μM respectively. The incubation of fibers with low Ca^{2+}, EGTA solution caused a reduction in the level of intracellular free calcium to 0.03 μM.
Partially supported by CONICIT S1-1148.

T-AM-Pos3 CORRELATIONS BETWEEN THE MUSCLE TYPE, pCa/TENSION RELATION, AND THE SDS GEL PATTERNS OF RABBIT SKINNED FIBERS. Schachat, F.*, Brandt, P.W., and B. Gluck. Department of Anatomy and Cell Biology, Columbia University N.Y. and *Anatomy Dept., Duke University, N.C.

pCa/tension curves were determined by the automated procedure of Brandt et al (1980) for skinned single fibers from three fast (psoas, plantaris, and tibialis ant.) and one slow (soleus) muscle. After the curves were run, fiber segments were subject to SDS gel electrophoresis (Schachat et al, 1981) and the tropomyosin, troponin and mysoin light chains characterized. Fibers from the soleus fell into three classes according to the light chain complements and to the pCa/tension curves. When only slow light chains were present on the gels two classes could be identified depending on the ratios of LCS1:LCS2. When this ratio was greater than 1 the pCa/tension curves had slopes (n: Hill factor) less than 2, and as the ratio fell below 1 the slopes (n) increased up to 3 or more. When the gels show major amounts of fast light chains the slopes of the pCa/tension curves were greater than 3. Gels of fast muscles showed only fast light chains and the pCa/tension curves were steep (n=4 to 6) with pKd's of 6.0.

T-AM-Pos4 **BIAXIAL CONSTITUTIVE RELATIONS OF PASSIVE CANINE MYOCARDIUM.** L. Demer, R. Strumpf, and F.C.P. Yin. Departments of Medicine and Biomedical Engineering, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

It has long been presumed that myocardium is intrinsically anisotropic and viscoelastic, but the requisite biaxial data to verify this are not available. We determined the biaxial passive, constitutive relations in thin (1-2mm) slices of canine myocardium with relatively uniform fiber orientation from left ventricular free wall. After preconditioning we stretched at equal rates in the fiber and cross-fiber directions over a 100-fold strain rate. We studied two pseudostrain-energy functions, W1=C*exp(A1*Ex**2+A2*Ey**2+A4*Ex*Ey)] & W2=A1*Ex+A2*Ey+C[exp(B1*Ex+B2*Ey)] where Ex and Ey are Green strains,and the A's,B's, and C are coefficients fitted by nonlinear least squares methods. Representative coefficients for a particular specimen from the outer portion of the posterior wall near the base, at the slowest and highest strain rates are:

slowest: W1[A1,A2,A4,C] = [23,13,18,6]; W2 [A1,A2,B1,B2,C] = [-16,-10,10,6,1.9]

highest: W1 [A1,A2,A4,C] = [40,22,37,5]; W2 [A1,A2,B1,B2,C] = [-15,- 7,15,8,1.2]

Both models fit the data comparably. Some specimens were isotropic; others markedly anisotropic between the fiber and cross-fiber directions. All demonstrated strain-rate dependence. The directionality of the anisotropy appeared to persist across strain-rates. Thus, passive uncoupled layers of canine myocardium exhibit viscoelasticity and varying degrees and directions of anisotropy. The properties of the intact wall depend not only on the intrinsic properties of each layer but also on the structural arrangement, geometry and coupling between these layers.

T-AM-Pos5 APPARENT SHORTENING OF A-BAND AND DISARRAY OF TERMINAL CROSS-BRIDGES DURING ATP-INDUCED CONTRACTION OF GLYCERINATED MUSCLE. Paul Dreizen and Lawrence Herman. Biophysics Graduate Program and Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, and Department of Anatomy, New York Medical College, Valhalla, NY.

Electron microscopic studies were done on longitudinal sections of glycerinated rabbit psoas in relaxing solution, rigor, and ATP-induced contraction. Average A-band length is 1.55μ in sarcomeres with length above 2.0μ, but ATP-induced contraction is accompanied by decrease of A-band length to approximately 1.3μ before the I-band is obliterated. Significantly, this change occurs as the A-band approaches the N-line, which becomes evident as a well-defined structure in shortened sarcomeres. The N-to-N distance is ca. 1.8μ at sarcomere length of 2.0μ, and decreases progressively as sarcomeres shorten further. Over the range from 1.9 to 1.5μ, there appears to be disorganization of the orderly arrangement of cross-bridges in the terminal part of the A-band, and also sharp changes in density along A-band edges. Optical diffraction of individual sarcomeres yields transforms which can be grouped into those with meridional reflection at 143A, and others with axial repeat as low as 130A. The significance (if any) of the short repeats is unclear at present. However, the evidence for apparent decrease of A-band length, along with constant cross-bridge interval, can be most simply interpreted in terms of disarray of terminal cross-bridges of thick filaments, presumably related to the shift of sarcomere structure from the hexagonal lattice of AI overlap zone to the tetragonal lattice of the I-band close to the Z-line, and also to the outreaching of cross-bridges towards actin binding sites as the filament lattice expands during shortening of sarcomeres.

T-AM-Pos6 EFFECTS OF CALCIUM ENTRY BLOCKERS ON MECHANICAL RESPONSIVENESS OF MAMMALIAN SKELETAL MUSCLES. Esther M. Gallant, Department of Pharmacology, Mayo Foundation, Rochester, MN 55905.

The importance of $[Ca^{2+}]_o$ in excitation-contraction (E-C) coupling, especially of mammalian skeletal muscle, has not yet been defined. Therefore, I studied the effects of several agents known to block the voltage-activated entry of Ca^{2+} into other types of cells to determine whether mechanical function was altered and whether a role for $[Ca^{2+}]_o$ in E-C coupling could be confirmed. Drugs compared were verapamil (V), the + and - stereoisomers of methoxyV (D600) and nifedipine. Membrane resting potentials and mechanical responses to electrical stimuli were recorded from murine EDL and soleus muscles. Twitch and tetanic force of both muscles was depressed 50% by 25 μM V after 30 min. Twitch kinetics were unaltered. Tetani faded markedly in the presence of V at high stimulation frequencies (200-300 Hz) but not at 100 Hz. Depression of twitches and tetani by V was the same after 30 min whether or not the preparation was stimulated at 0.05 Hz. All effects reversed slowly on removal of V. Some tetanic fade was evident with as little as 1 μM V. Surface membranes were depolarized slightly (4mV) with V. Nifidepine had no effect on either force or membrane potential. Decreased $[Ca^{2+}]_o$ did not alter responses to V. Increased $[Ca^{2+}]_o$ (which by itself had no effect) resulted in less tetanic force and more fade than V alone. Caffeine contractures of EDL (but not soleus) were depressed and slowed by V. Increased Ca^{2+} reversed this effect. Both + and - D600 depressed force slightly less than V and depolarized about the same amount. The actions of V and D600 are apparently complex. Block of Ca^{2+} entry may be responsible for depression of caffeine contractures, but other mechanisms must be involved in depression of twitches and tetani. This is consistent with voltage-activated Ca^{2+} entry having a role only in contractures. (Supported by AHA, Minnesota Affiliate and MDA.)

T-AM-Pos7 COMPARISON OF SKELETAL MUSCLE MYOSIN LIGHT CHAIN KINASES BY ANTIBODY CROSSREACTIVITY. M.H. Nunnally and J.T. Stull. Dept. Pharmacol., Univ. TX Hlth. Sci. Ctr., Dallas, Texas 75235.

Antibodies to rabbit skeletal muscle myosin light chain kinase (MLCK) have been used to compare MLCK in several animal species and muscle types. In rabbit skeletal muscle (slow- and fast-twitch fibers) this antiserum only recognizes a polypeptide of 90,000D, as determined by antibody overlay of proteins electrophoretically transferred to nitrocellulose paper from SDS-polyacrylamide gels. There is no indication that the 90K polypeptide is a breakdown product of a larger protein, either in low-salt extracts or myofibrillar pellets of rabbit skeletal muscle.

This antiserum inhibits the catalytic activity of skeletal muscle MLCK as measured by the incorporation of ^{32}P into isolated myosin P-light chains. Similar antibody dilution curves for inhibition of MLCK activity in extracts were observed for all animal species (rabbit, rat, mouse, dog, cat, beef and chicken) and different fibers (slow-twitch oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolytic) tested.

By use of the antibody overlay technique, we have observed that the molecular weight of MLCK is constant within a given animal species, regardless of skeletal muscle fiber type. However, there are differences among the species. The MLCK detected in dog skeletal muscle extracts is a polypeptide of ∿100,000D. The mouse and rat MLCK are ∿75,000D, while the guinea pig MLCK is ∿80,000D. The antiserum does not react with MLCK in rabbit smooth or cardiac muscle extracts.

These results demonstrate that the molecular weight of MLCK in mammalian skeletal muscle can vary among different animal species. The range of molecular weights for skeletal muscle MLCKs (75-100K) is smaller than the reported values for smooth muscle MLCK (130-150K).

T-AM-Pos8 TEMPERATURE DEPENDENCE OF TENSION PRODUCTION BY ATRIAL TRABECULAE. P.J. Reiser and B.D. Lindley, Dept. of Physiology, Case Western Reserve Univ., School of Medicine, Cleve., Ohio 44106.

Frog (Rana pipiens) atrial trabeculae (diam. 50-150 μm) were activated by a variety of means including exposure to 100 mM KCl Ringer solution, electrical stimulation at 10, 20 or 60 Hz (high current, sine- or squarewaves), and exposure of chemically skinned (i.e., Brij-58 treated) trabeculae to solutions with varied pCa values. In addition to performing experiments at a variety of steady temperatures (T), T-jump experiments involved rapid flush or brief microwave heating to change the T several degrees in a fraction of a second. The steady T experiments indicate a roughly parabolic dependence of high [K] contracture tension on T between 8°C and 30°C with a tension minimum occurring around 20°C if the contracture solution contains Na, similar to the results of Chapman, 1973 (J. Physiol., 231:233-249). However, with low $[Na]_o$, there is a steady increase in the high [K] contracture tension with T in the same T range. In the T-jump experiments, using high [K] solution or electrical stimulation to activate the trabeculae, heating results in a potentiation of tension of 5 to 10%. Demonstrating a minimum in the T-dependence requires a period of T equilibration and the presence of Na in the contracture medium and may reflect shifts in the relative contribution of intra- and extracellular calcium to activation. The immediate effect of an increase in T is to increase force provided a substantial level of activation is present. Supported by USPHS (HL 19848) and Northeast Ohio Affiliate, American Heart Association.

T-AM-Pos9 ISOMETRIC FORCE DEVELOPMENT IN SKELETAL MUSCLE CELLS OF THE FROG DURING MAINTAINED EXPOSURE TO DEUTERIUM OXIDE (D_2O). Jack A. Rall and D.G. Allen. Department of Physiology, University College London, London, England.

When muscle cells are incubated in D_2O-Ringer, tetanus force decreases then recovers to values slightly greater than those observed in H_2O-Ringer (Cecchi et. al. J. Physiol. 317, 207, 1981). Further study of this phenomenon was undertaken with single cells (n = 5) from tibialis anterior muscles of R. temporaria at 10°C. Upon exposure to D_2O-Ringer, tetanus force abruptly decreased to near zero and then slowly recovered to a variable extent over the next hour. Maximum recovery of force in D_2O-Ringer ranged from 8 to 110% of the peak force produced in H_2O-Ringer (sarcomere length of 2.2 um). During exposure to D_2O-Ringer, kinetics of the mechanical responses slowed at first and then accelerated with time but not back to the values observed in H_2O-Ringer. If the cell was exposed to new D_2O-Ringer during this period, force decreased back toward zero and then recovery proceeded with a time course similar to that observed during the first exposure to D_2O-Ringer. Recovery of force in D_2O-Ringer could not be attributed to contamination of D_2O by H_2O. This conclusion is based upon the density of D_2O-Ringer which was measured after maximum recovery of force (110% of value in H_2O-Ringer) compared to the density of H_2O-Ringer. All effects were reversed upon return to H_2O-Ringer. The mechanism of variable recovery of force in D_2O-Ringer and of temporary reversal of force by replacement with new D_2O-Ringer is unknown. (Supported, in part, by NIH grant AM20792.)

T-AM-Pos10 QUANTITATIVE ANALYSIS OF ATP TURNOVER IN RELATION TO CA^{2+}-ACTIVATED TENSION IN CHEMICALLY SKINNED GUINEA PIG TAENIA COLI. Per Hellstrand and Anders Arner. Dept of Physiology and Biophysics, University of Lund, Sweden (Intr. by J.M. Krisanda).

The activity of the contractile system in muscle is reflected in various aspects by force, shortening velocity and ATP turnover (J_{ATP}). In taenia coli skinned in 1 % Triton X-100 we measured J_{ATP} simultaneously with isometric force in the presence of MgATP (3.2 mM), phosphoenolpyruvate (5 mM), pyruvate kinase (20 U/ml), calmodulin (1 μM) and NaN_3 (1 mM) at 22°C. ADP release during 5 - 10 min intervals was measured by NADH-linked fluorimetric assay. In relaxed preparations (pCa = 9) J_{ATP} was 0.45 ± 0.05 μmol/min·g (n = 8) and increased on activation in a dose-related manner to a maximum at pCa = 4.5 of 1.23 ± 0.07 μmol/min·g at a force of 30.2 ± 1.8 mN/mm^2. Between pCa 5.75 and 4.5 force increased by 17 % but suprabasal J_{ATP} by 113 %. This Ca^{2+}-dependent metabolic activation may be the result of increased rate of cross-bridge turnover with little change in maintained tension, but it could also reflect activation of ATPase unrelated to cross-bridge activity. To resolve this question muscles were activated at pCa 4.5 or 5.75 both at optimal length and at short length where no active tension was produced. J_{ATP} at the short length at pCa = 4.5 was more than twice that of relaxed muscles and significantly higher than at pCa = 5.75. Tension-related J_{ATP} tended to be higher at pCa = 4.5. The results suggest a tension-related J_{ATP} reflecting rate of cross-bridge turnover dependent on $[Ca^{2+}]$. At high $[Ca^{2+}]$ there is also a significant contribution from non-tension dependent J_{ATP}. Supported by Swedish MRC (00028).

T-AM-Pos11 REGULATION OF MYOSIN ISOZYMES IN RAT VENTRICULAR MUSCLE BY CATECHOLAMINES. G. McClellan, S. Winegrad, M. Tucker, R. Horowits and L-E Lin. School of Medicine, University of Pennsylvania, Philadelphia, PA

Hyperpermeable cells have been prepared from rat right ventricular trabeculae by an overnight soak in 10 mM EGTA solution (McClellan and Winegrad, J.G.P. 75, 283, 1980). Maximum Ca activated force (contractility) is increased an average of 250% by exposing the fibers to a combination of 1 μM cAMP, 5 mM theophylline, 1% Triton X-100 in a relaxing solution. The degree to which contractility is increased depends on the relative concentration of the V_1 isozyme of myosin. When the concentration of V_1 is decreased by thyroidectomy, aging or hypertrophy from greater afterload, the amplitude of the increase in contractility declines in parallel. After almost complete conversion to the V_3 isozyme 5 weeks after thyroidectomy, cAMP with theophylline and detergent does not increase contractility. Activators of the β adrenergic system such as epinephrine and GTP also increase contractility in proportion to the amount of V_1 present. Alpha adrenergic agonist phenylephrine together with 1% Triton X-100 in relaxing solution increases contractility in proportion to the relative amount of V_3 myosin that is present. In young euthyroid hearts, 0.3 μM phenylephrine increased contractility by almost 60%. Following thyroidectomy the size of the increase in contractility became larger in parallel with the relative increase in V_3, and 5 weeks after thyroidectomy phenylephrine increased contractility by 240%. These results indicate that the maximum Ca activated force can be increased by the adrenergic system. Beta adrenergic agonists appear to enhance contractility of force generators involving V_1 myosin and alpha agonists enhance contractility where V_3 myosin is involved. Supported by N.I.H. Grant HL 16010.

T-AM-Pos12 REGULATION OF ATPASE OF MYOSIN AND ACTOMYOSIN IN RAT HEART BY CATECHOLAMINES. S. Winegrad, A. Weisberg, G. McClellan, and L-E Lin. School of Medicine, University of Pennsylvania, Philadelphia, PA

A histochemical procedure has been developed for measuring the Ca activated ATPase activity of the V_1 isozyme of myosin in the presence of V_3 myosin in rat ventricular muscle (Weisberg et al, Circul. Res. Dec. 1982). The technique has been used to measure the change in concentration of V_1 following thyroidectomy and it is sufficiently sensitive to detect differences in the concentration of V_1 as little as 12%. The same procedure has been applied to measure differences in the Ca ATPase activity at a constant concentration of V_1 myosin when the degree of catecholamine stimulation is varied. Young, male, litter mate rats with over 90% V_1 myosin in the ventricles were injected intravenously with 100 mg 6-OH-DOPA/kg body wt. to deplete the tissues of catecholamine. Animals were sacrificed 1, 3, 5 and 18 hrs. after injection in order to follow the ATPase activity during the early period when massive release of catecholamine results in stimulation as well as the later period when depletion has been completed. The hearts were isolated, frozen in isopentane cooled with liquid N_2 and then sectioned at 6 μ on a cryostat. The sections were dried, incubated at pH 10.5 to inhibit V_3 myosin and then incubated with ATP for 37°C for 10 min. according to a modification of the Padykula-Herman procedure. ATPase activity rose markedly during the first 3 hours after injection and then fell to a value below control by 18 hours. Histochemical assay of actomyosin ATPase in serial sections of the same hearts showed a parallel rise and fall in activity following administration of 6-OH-DOPA. These results indicate that the enzymatic properties of the contractile proteins are directly regulated by catecholamines. Supported by N.I.H. Grant HL 15835.

T-AM-Pos13 MEASUREMENT OF THE Ca^{2+} GRADIENT ESTABLISHED BY THE Ca^{2+}-ATPase OF BOVINE SARCOLEMMA Deborah Dixon, Dept. of Pharmacology, Univ. of Miami School of Medicine, P.O. Box 016189, Miami, Fl. 33101.

The kinetics of ATP-energized Ca^{2+} pumping by fragmented (vesicularized) bovine sarcolemma (SL) were measured using a fluorescent probe technique and with standard isotope techniques. Active uptake was initiated by addition of 1 mM Mg-ATP to 5.5 ug/ml SL in a medium containing 160 mM KCl, 20 mM Mops/Tris pH 7.4 and 0.5 mM Ca^{2+}-EGTA buffer, at 37°C. Active uptake occurred as a slow process with a $t_{\frac{1}{2}}$ of ∿3 min. Its kinetics were studied by millipore filtration or, more conveniently, by monitoring the increase in fluorescence of chlorotetracycline (CTC). The CTC fluorescence could be accurately calibrated in situ, allowing transformation of the fluorescence data into internal Ca^{2+} concentrations. This was possible because extremely slow rates of Ca^{2+} passive equilibration were observed. The initial rate and maximal Ca^{2+} uptake at steady-state both showed a sigmoidal dependence on $[Ca^{2+}]_o$, with a K_m value of ∿6 x 10^{8}M at $[CaEGTA]/[EGTA]_t$ = 0.7. A maximal internal free concentration of ∿34 + 1.3 mM was determined using the CTC method. This corresponds to a value of ∿81 ± 5 nmole/mg $^{45}Ca^{2+}$, determined under identical conditions in the absence of CTC. The latter number was used to calculate a total internal (free plus bound) Ca^{2+} concentration of ∿37 mM, using sucrose entrapment as a measure of the internal volume (µl/mg). The estimation of the internal passive Ca binding capacity is equivalent to ∿3 mM. All of the accumulated Ca^{2+} was releasable over a several minute period by the addition of 160 mM NaCl. A Ca^{2+} gradient of 2.8 x 10^4 was measured for $[Ca^{2+}]_i = K_m$. The implications of these findings for the pump mechanism and cellular homeostasis will be discussed.

T-AM-Pos14 EFFECT OF REDUCING EXTERNAL K^+ CONCENTRATION ON INTERMEDIARY METABOLISM AND ISOMETRIC FORCE IN RAT ANOCOCCYGEUS SMOOTH MUSCLE. S. Davidheiser, J. Joseph, and R. E. Davies. Dept of Animal Biology, Sch. of Vet. Med., University of Pennsylvania, Phila., PA 19104.

The effect of reducing the external K^+ concentration on aerobic lactate production (J_{lac}), oxygen utilization (J_{O2}), and isometric force (P) was determined in isolated rat anococcygeus smooth muscle (ASM). These muscles are innervated by adrenergic nerves and maintain sustained tonic contractions when stimulated. It has been reported that in hog carotid artery aerobic J_{lac} was related to activity of the Na^+-K^+ pump, and J_{O2} was correlated with isometric force (Paul, et al., Science, 1979). ASM incubated in K^+-free medium at 37° spontaneously developed force that reached 60% of maximum force (P_o) by 90 min. (P_o obtained by stimulation with 10^{-5}M norepinephrine.) J_{lac} and J_{O2} both increased above resting levels (J_{lac} resting= 0.232±S.E. 0.026 µmoles/g/min, n=15; J_{O2} resting= 0.414±0.052, n=7) and remained constant between 30 and 60 min. Steady state J_{lac} and J_{O2} during this period expressed as fractions of the resting values were 3.62±0.37, n=12 and 1.86 ±0.12, n=4, respectively. P/P_o was 0.36±0.10, n=10. Force production in ASM treated with the α-adrenergic blocking agent phentolamine (10^{-5}M) was significantly reduced to 0.064±0.032, n=5. This indicated that the P responses were due to release of endogenous norepinephrine. J_{lac} increased to levels similar to those in muscles incubated in K^+-free medium alone, but J_{O2} was not related to J_{lac}. In three muscles treated with phentolamine and incubated in K^+-free medium, $J_{O2}/J_{O2 resting}$ was not significantly different from 1.0 (0.89, 1.05 and 0.88). In ASM, unlike vascular smooth muscle, J_{lac} increased under conditions where the Na^+-K^+ pump was inhibited. J_{O2}, but not J_{lac}, was related to isometric force production. (Supported by NIH Grant HL 15835.)

T-AM-Pos15 Shortening Heat and Work Production --- The Effect of Distance Shortened and Velocity of Shortening. Takenori Yamada and Earl Homsher, Department of Physiology, School of Medicine, University of California at Los Angeles, Los Angeles, Calif. 90024, U.S.A.

Shortening heat and work production were measured in 2.1 sec tetani in Rana pipiens sartorius muscles. After 1.0 sec of isometric stimulation, the muscles shortened distances ranging from 0.015 to 0.5 micron per sarcomere (all ending at a sarcomere length of 2.05 micron). The velocities of shortening of V_{max}, 1/2 V_{max} and 1/4 V_{max} were used. The amount of shortening heat and work produced were measured in the 1.0 to 2.1 sec interval of the tetanus. Both the shortening heat and work production were plotted against the distance shortened, and both plots found to be composed of a linear and a non-linear component. The linear component of shortening heat and work was 21.1 and 7.9 mJ/g/sec at V_{max}, 20.3 and 20.9 mJ/g/sec at 1/2 V_{max}, and 13.3 and 22.9 mJ/g/sec at 1/4 V_{max}, respectively. The non-linear component of shortening heat and work was determined from the y-intercept of a straight line fitted to the linear component, and was 2.9 and 2.8 mJ/g at V_{max}, 1.5 and 2.2 mJ/g at 1/2 V_{max}, and 0.9 and 1.6 mJ/g at 1/4 V_{max}, respectively. Significant part of the non-linear energy production cannot be explained by the known load-dependent shortening heat production and the series elastic work performed by actomyosin. (Supported by USPHS grant AM309881 and the MDA).

T-AM-Pos16 **31-PHOSPHORUS NMR SATURATION TRANSFER EVIDENCE FOR THE PHOSPHOCREATINE-ATP ENERGY TRANSPORT SHUTTLE IN INTACT PERFUSED RABBIT HEARTS.** M.V. Miceli, J.A. Hoerter*, W.E. Jacobus, Medicine and Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Current models of energy transduction in heart recognize the importance of creatine kinase in supplying high energy phosphate to meet the energy demands of contraction and relaxation. To more fully elucidate the role of creatine kinase in intracellular energy transport, we employed ^{31}P NMR saturation transfer to measure the rate of chemical exchange from phosphocreatine (PCr) to ATP in perfused rabbit hearts. If the energy transport hypothesis is valid, then the rate of this exchange (k_2) should should parallel changes in mechanical performance. Hearts were isolated from 1-2 kg female rabbits, and perfused retrograde with phosphate-free Krebs Ringer bicarbonate buffer bubbled with 95% O_2 + 5% CO_2 (pH 7.45) at 37°C. Coronary flow was maintained by constant pressure or by peristaltic pumping. Isovolumic left ventricular developed pressure (LVDP) was measured with a fluid filled latex balloon positioned in the left ventricle, and connected via a catheter to a Statham P 23 Db pressure transducer. Heart function (LVDP X heart rate) was varied from 0 (tetracaine, 150 mg/l) to 30 X 10^3 by varying perfusate [Ca^{2+}] (0.1 mM to 2.5 mM). Pacing rate was also varied at constant [Ca^{2+}]. Functionally dependent changes in k_2 (0.6 to 1.3 sec^{-1}) were exhibited with varied calcium. In paced hearts k_2 showed a dependence on the pacing rate above 200 beats per minute. The changes in k_2 as a function of myocardial performance support the phosphocreatine energy transport model.

T-AM-Pos17 **INTRACELLULAR CALCIUM ALTERS HIGH ENERGY PHOSPHATES AND INTRACELLULAR pH. A ^{31}P NMR STUDY OF THE ISOLATED RAT HEART.** J.A. Hoerter*, M.V. Miceli, D.G. Renlund*, G. Gerstenblith*, E.G. Lakatta*, and W.E. Jacobus, Medicine and Physiological Chemistry, the Johns Hopkins University School of Medicine and the Gerontology Research Center, Baltimore, MD 21205.

^{31}P NMR was used to measure changes in the high energy phosphates and intracellular pH (pH_i) induced by altering intracellular calcium content (Ca_i). Pulsed Fourier transform spectra were obtained from isolated isovolumic rat hearts perfused with constant flow at 37°C with phosphate-free Krebs Ringer bicarbonate buffer (glucose = 16 mM; pH_o = 7.45). Five min. following a reduction in perfusate K^+ (K_o) from 5.9 to 0.0 mM (increased Ca_i), resting pressure increased more than 5 fold, oxygen consumption increased by 15%, phosphocreatine (PCr) and ATP were markedly decreased, and pH_i decreased from 7.20 to 6.90. Similar metabolic alterations occured after the addition of oubain to control perfusate. These changes in ATP and PCr suggest that the rate of ATP synthesis does not match the presumed increased rate of utilization required to maintain high resting pressure. The decreased rate of ATP synthesis could be the result of increased oxygen linked mitochondrial Ca^{2+} translocation, which is known to occur in preference to ATP production. Such an effect would also produce protons, resulting in intracellular acidosis. Lowering Ca_o from 2.5 to 0.08 mM abolished contractility, decreased oxygen consumption, and increased both PCr and pH_i. These latter metabolic effects are presumably due to decreased ATP utilization, as similar results were obtained when mechanical activity was abolished by tetracaine. Overall, these results show that alterations in Ca_i induce marked metabolic abnormalitites.

T-AM-Pos18 **REGULATION OF HEART MITOCHONDRIAL RESPIRATION BY [CREATINE] AND [PHOSPHOCREATINE].** W.E. Jacobus, and D.M. Diffley* (Intr. by P.L. Pedersen), Medicine and Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Defining how extramitochondrial metabolites influence rates of heart mitochondrial respiration is essential for understanding the control of myocardial high energy phosphate production. When coupled to oxidative phosphorylation, the production of phosphocreatine (PCr) from creatine (Cr) by mitochondrial creatine kinase (CK_m) conforms to the balanced equation: Cr + Pi = PCr + H_2O. This suggests that the rate of the forward CK_m reaction, and thus oxygen consumption, may be regulated by [Cr], [PCr], or [Pi], alone or in combination. The effects of altering these metabolites upon mitochondrial rates of respiration were examined in vitro. Rat heart mitochondria were incubated in succinate containing oxygraph medium (pH 7.2, 37°C) supplemented with 5 combinations of [Cr] (1.0 to 20 mM), [PCr] (0 to 25 mM), and [Pi] (0.25 to 5.0 mM). The CK_m reaction was started by additions of 0.5 mM ATP. Under physiological conditions, with constant [Cr] (5.0 mM), increases in Pi or decreases in PCr had little influence upon the rates of respiration. However, when Pi was held constant at 2.0 mM, the measured rates of respiration clearly became dependent upon changes in [Cr] and [PCr]. Initially, respiratory rates increased as a function of increases in [Cr]. However, as PCr fell below 10 mM, product "deinhibition" was observed, and respiration increased markedly. These results support the view that increasing [Cr] and decreasing [PCr] may be primary cytoplasmic parameters regulating heart mitochondrial oxidative phosphorylation, and thus the rates of myocardial oxygen consumption.

T-AM-Pos19 PROPORTIONALITY BETWEEN RESPIRATORY RATE AND CREATINE PHOSPHATE (CP) LEVEL IN FROG SKELETAL MUSCLE. Michael Mahler, Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles 90024.

After a contraction of 0.2-1 sec in the isolated sartorius of R. pipiens at 20°C, the suprabasal rate of O_2 consumption (ΔQ_{O2}) rises rapidly to a peak, then falls monoexponentially (τ = 2.6 min). Since [ATP] stays essentially constant during this time, the end product of oxidative phosphorylation is CP, and the kinetics of ΔQ_{O2} imply that $\Delta Q_{O2}(t) = (-1/\tau \cdot p) \cdot \Delta[CP]_t$, where $\Delta[CP]_t$ is the change in [CP] from its basal level at time t, and p is the P:O_2 ratio. As reported previously (Fed. Proc. 41:979,1982), this prediction has survived direct experimental tests employing tetani of up to 1 sec. As a further test, muscles were tetanized for 3.2, 5, or 10 sec, and $\Delta Q_{O2}(t)$ was measured for as long as the muscles remained oxygenated. The peak value of ΔQ_{O2} could always be determined, and in companion experiments Δ[CP] was measured under identical conditions. During a 10 sec tetanus, tension fell to 40% of its initial value, and ≈60% of the resting CP (23 μmole/g) was split; ΔQ_{O2} subsequently rose to >25 times the basal Q_{O2}. After normalization by total creatine, regression of $\Delta Q_{O2}(t_{peak})$ (μmole/min·g) on $\Delta[CP]_{tpeak}$ (μmole/g) for tetani of 0.2-10 sec gave Y = (-0.000336±0.00105)-(0.0645±0.0050)X, r=0.99, in good agreement with the predicted proportionality ($1/\tau \cdot p$) = 0.062). This relationship differs drastically from that predicted by the assumption that Q_{O2} is determined by cytoplasmic [ADP] and [ATP], via the ATP:ADP translocase, and that the cytoplasmic mass action quotient for creatine kinase (CK) stays always very near its equilibrium value. It seems plausible that the major cytoplasmic signals controlling Q_{O2} in muscle are [creatine] and [CP], and that the changes in [ADP] to which the translocase responds are largely restricted to a microenvironment near mitochondrial CK and translocase. (Supported by AHA Senior Investigatorship.)

T-AM-Pos20 Temperature Induced Alterations in Intracellular pH of Unanesthetized Salamanders. B.M. Hitzig, C.T. Burt, and H. Kazemi, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

High resolution (109 MHz) Phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR) was performed on unanesthetized newts (bimodal breathers, lungs and skin), and redbacked salamanders (lungless, use only skin for gas exchange) at 22° (n=10) and 10°C (n=10). Inorganic phosphate (P_i), phosphocreatine (PCr), sugar phosphate, and ATP peaks were easily identified. Intracellular pH (pHi) was calculated by comparing the chemical shift of the observed Pi peaks relative to PCr, after the animals had reached a steady state, to the NMR titration curve of a known standard. The tissue within the coil was confined to the tail of the animal and characteristic muscle spectra were obtained. The spectra revealed 2 peaks at the normal position for Pi. Processing the data using convolution difference spectra more clearly demonstrated the presence of 2 Pi peaks. Breathing 100% oxygen resulted in the disappearance of both Pi peaks and a concomitant increase in PCr demonstrating that the Pi peaks are derived from a single cell population, muscle. Nitrogen breathing caused the Pi peaks to collapse into one large peak. These data indicate the 2 peaks represent Pi pools of 2 separate compartments (probably cytosol and mitochondria) within the muscle cells. At 22°C pHi was the same in both animals; however, changing body temperature to 10°C resulted in a marked alkalinization (+0.017 units/°C in newts, and +0.031 units/°C in red backs). These results demonstrate that pHi in intact animals is essentially independent of mode of gas exchange and is mainly dependent upon body temperature supporting the alpha-imidazole hypothesis of intracellular acid-base regulation.

T-AM-Pos21 CHEMICAL ENERGY USAGE DURING ACTIVATION AND STRETCH: A PROBE OF CROSSBRIDGE-RELATED ENERGY USAGE IN MAMMALIAN SMOOTH MUSCLE. M.J. Siegman, T.M. Butler and S.U. Mooers. Dept. of Physiology, Jefferson Med. Coll., Thomas Jefferson Univ., Philadelphia, Pa 19107

High-energy phosphate usage ($\Delta\sim P$) was determined directly as changes in phosphocreatine and adenine nucleotide contents of rabbit taenia coli in which respiration and glycolysis were inhibited. During an isometric tetanus in 1.9 mM Ca^{++}-Krebs medium at 18°C, the average rate of $\Delta\sim P$ is 4x higher during force development than maximum force maintenance. Similar differences in the magnitude of Vmax (slack test) were observed. An activated stretch (93 to 98%L_o, 0.12L_o/min) during the period of force development decreased the $\Delta\sim P$ from 7.6±0.8 to 3.6±0.8 mmole/(mole C_t·sec), where C_t=total creatine=2.7 μmole/g, but had no detectable effect during the period of force maintenance. Activated stretch apparently slows the ATP utilization due to actin-myosin interaction, hence reducing energy usage to a greater extent when the crossbridge cycling rate is high. The energy demand during isometric force maintenance is therefore largely due to reactions other than actin-activated myosin ATPase. In 4.5 mM Ca^{++}-Krebs the $\Delta\sim P$ for isometric force development doubled while maximum active force increased only 14±5%. Activated stretch during this period reduced the energy usage by 80% compared to isometric conditions. Thus, the increased calcium concentration led to a 3x increase in crossbridge cycling rate and a slight increase in active force. Similar degrees of myosin light chain phosphorylation (MyLCP) at the end of the force development phase were observed in 1.9 and 4.5 mM Ca^{++}-Krebs. The apparent increase in crossbridge cycling rate in 4.5 mM Ca^{++} is probably not mediated through MyLCP. The ability of activated stretch to decrease $\Delta\sim P$ from crossbridge cycling may serve to regulate chemical energy usage in smooth muscle of hollow organs where stretch during the initial period of activation is common. (Supported by HL15835 to Pennsylvania Muscle Institute)

T-AM-Pos22 ^{31}P AND ^{13}C NMR ANALYSIS OF GLYCOLYSIS IN EHRLICH ASCITES TUMOR CELLS. R.J. Gillies, Dept. of Biochemistry, Colorado State University, Ft. Collins, Colo. 80523

Wide-bore, 360 MHz NMR spectroscopy was used to monitor intracellular pH (pH^{in}), inorganic phosphate concentrations (Pi^{in}) and glycolytic rates in suspension of Ehrlich ascites tumor cells.

Preliminary data has been obtained using stirred cell suspensions. Under conditions of endogenous respiration, pH^{in} is approximately 7.2 and Pi^{in} is approximately 6 mM. Addition of glucose (20-50 mM) to this aerobic suspension precipitates a drop in pH^{in} to ~7.0 and a decrease in Pi^{in} to ~2 mM. As determined by ^{13}C NMR, glucose is consumed at a rate of 0.33 (μmoles-min^{-1} · 10^{-8} cells) and lactate is produced at a rate of 0.08 under these conditions. Switching the gas supply from 95%O_2:5%CO_2 to 95%N_2:5%CO_2 precipitates a further drop in pH^{in} and a rise in Pi^{in} to ~7 mM. Glucose consumption under anaerobic conditions is ~0.54 and lactate production is ~0.45. These data indicate that higher glycolytic rates are accompanied by increased Pi^{in} and decreased pH^{in}.

A problem with the interpretation of these data is that the metabolism is not steady-state due to the static nature of the suspensions. Lactate accumulation contributes to significant pH drift and may cause the lowered pH^{in} values obtained. To circumvent this problem, a lattice of hollow fibers has been designed which will facilitate waste product removal and will allow the use of more physiological glucose levels. Data will be presented from studies employing this new apparatus.
Supported by NIH-AM-27121 and NIH/BRSG SO7-RR07127.

T-AM-Pos23 *IN VIVO* P-31 NMR MEASUREMENTS OF EXERCISE METABOLISM OF THE LIZARD *ANOLIS CAROLINENSIS*. Paul G. Schmidt and E. Norbert Smith, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

P-31 NMR at 121.5 MHz was used to measure levels of ATP, creatine phosphate (CP), Pi and sugar phosphates in lizards before, during and following strong exercise as a function of ambient temperature. For these experiments the tail and rear legs of the unanesthetized animal were lightly taped together and passed through the sideways mounted, solenoid design, NMR coil. Subcutaneous Ag/AgCl electrodes were placed for monitoring movement and applying electrical shocks for stimulation. NMR accumulation times of 5 or 10 min. provided adequate signal-to-noise ratios. Prior to stimulus, CP levels were highest (57% of the total) for the metabolites observed; their relative concentrations did not change significantly with temperature over the range 5° to 40°C. During 10 minute bouts of exercise, CP levels dropped to less than 1/2 initial values, and Pi increased accordingly. ATP levels remained approximately constant. Rates of change of CP during exercise and recovery were temperature dependent, being greatest at the preferred temperature (30 - 33°) and declining at higher and lower temperatures. A new method is introduced here for absolute quantitation of phosphorus metabolites in NMR experiments which uses the rates of change of signal areas as its basis.

T-AM-Pos24 ^{13}C AND ^{31}P NMR AND ESR STUDIES OF GLUCOSE METABOLISM IN STAPHYLOCOCCUS AUREUS. F. S. Ezra, D. S. Lucas and A. F. Russell, The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

^{13}C and ^{31}P NMR spectra were obtained from glycolyzing S. aureus cells grown in BHI. The ^{13}C NMR spectra of intact cells incubated with [1-^{13}C] or [6-^{13}C] glucose at pH 6.0 show resonances due to mannitol, lactate and ethanol as the major end-products of glycolysis. At pH 7.5, the amount of mannitol relative to lactate is significantly reduced. This reflects an enhanced PFK activity at the higher pH. The resonances due to the intra- and extracellular lactate are well-resolved and can be monitored during the course of glycolysis. Upon oxygenation, the uptake of lactate by the cells is readily observed.

The intracellular lactate and orthophosphate resonances in the ^{13}C and ^{31}P NMR spectra tend to broaden and shift downfield during anaerobiosis. These trends in linewidths and chemical shifts are reversed when the cells are oxygenated. Moreover, the ESR spectrum of a perchloric acid extract shows a sextet characteristic of manganese hexaquo ions. The NMR results, therefore, are attributed to a binding of the manganese ions to the intracellular lactate and orthophosphate. In-vivo, the magnitude of the ESR signal is dependent upon the metabolic and oxygenation state of the cells. Possible mechanisms by which manganese is involved in the metabolism of oxygen will be discussed.

T-AM-Pos25 2-D NMR SPECTROSCOPY OF PERFUSED RAT HEARTS Christopher J. Turner and Pamela B. Garlick (Intr. By Richard B. Robinson) Department of Chemistry, Columbia University New York, NY 10027 and Department of Pharmacology Columbia University College of Physicians and Surgeons, New York, NY 10032.

2-D NMR techniques have been applied to the study of phosphorus-containing metabolites in the isolated perfused rat heart. 2-D magnetization exchange spectroscopy (Jeener et al. J. Chem. Phys. 71 4546 (1979)) has been used to monitor the in situ exchange reaction catalysed by the enzyme creatine kinase: $MgATP^{2-}$ + creatine $\rightleftharpoons$ $MgADP^{-}$ + phosphocreatine + H^{+}. The off-diagonal (or cross) peaks correlating the γ-ATP and phosphocreatine resonances were of the same intensity as the diagonal (or auto) peak of γ-ATP when the value of the mixing time was set to 0.4s. 2-D spectroscopy (Aue et al. J. Chem. Phys. 65 4226 (1976)) has been used to resolve the multiplet structure of the α- and γ-ATP resonances, whose natural linewidths have been determined to be approximately 12 Hz. No multiplet structure of the β-resonance has been detected. We suggest that this is because the linewidth of the β-peak is comparable with its phosphorus spin-spin coupling constant (∿17 Hz). This suggestion of a greater linewidth of the β-peak compared with the α- or γ-peaks is supported by the decrease in the intensity ratios of β:α ATP and β:γ ATP in the echo experiments as compared to the ratios in conventional one-dimensional spectra. We believe that 2-D NMR techniques may thus provide alternative ways in which to probe both enzyme kinetics and the state of the ATP in the intact heart. (Supported by NSF and NIH #HL-27318).

T-AM-Pos26 DIRECT OBSERVATION OF THE MITOCHONDRIAL PHOSPHATE IN PERFUSED HEARTS BY ^{31}P NMR. Truman R. Brown, Pamela B. Garlick, Richard H. Sullivan and Kamil Ugurbil (Intr. by Brian F. Hoffman, Dept. of Pharmacol.) Bell Labs, Murray Hill, NJ 07974 and Depts. of Pharmacol. and Biochem., Columbia University College of Physicians and Surgeons, New York, NY 10032.

^{31}P NMR measurements were made on rat hearts perfused by the Langendorff method with phosphate-free Krebs-Henseleit buffers at various pH's (obtained by lowering the bicarbonate concentration). At all pH's, two inorganic phosphate (P_i) peaks were observed in spectra from hearts whereas only a single peak was seen in the PCA extract spectra. The smaller, more alkaline P_i peak seen in the heart spectra was identified as coming from the phosphate in the mitochondrial matrix for the following reasons: (1) Infusion of valinomycin into the heart (at a final concentration of $5.10^{-9}M$) resulted in a large increase in the size of this peak but no shift in its position, as predicted from experiments with isolated mitochondria; (2) No shift of this peak was observed on lowering the buffer pH to 6.9 or 6.6, thus eliminating the possibility that the peak was due to phosphate in the extracellular space (In contrast, the cytosolic pH shifted to 6.8 and 6.7 respectively, in these experiments.); (3) pH values obtained for isolated mitochondria indicate that the pH of these organelles is about 0.5 units more alkaline than the cytosol; these numbers agree with our determinations of the pH's experienced by the two P_i signals (7.45 and 7.05); and (4) The relative volumes of the matrix and cytosolic compartments calculated from the relative P_i intensities and the ΔpH across the mitochondria are in agreement with literature values.

T-AM-Pos27 ^{31}P-NMR SPECTROSCOPY OF SMOOTH MUSCLE. P.F. Dillon, R.A. Meyer‡ M.J. Kushmerick, Dept. of Physiology and Biophysics, Harvard Medical School, Boston, Mass. 02115, and T.R. Brown‡ Bell Laboratories, Murray Hill, N.J. 07974.

Perfused rabbit urinary bladders were reversibly stimulated by (ED50) $10^{-5}M$ acetycholine and 3x $10^{-6}M$ carbachol, producing length-dependent force generation. ^{31}P-NMR spectra, obtained at 109.3 MHz, contained seven peaks corresponding to Pi, PCr, γ-, α-, and β-ATP, and two unidentified peaks, one in the diester region and one in the monoester region. This peak is not G6P, G1P, or F6P. PCr/ATP ratio was 0.7 by both NMR and enzymatic analysis. The intracellular pH was 7.2. Upon carbachol stimulation, there was a reversible dose-dependent fall in PCr and pH, a rise in Pi, and no change in ATP. Ischemia accentuated these effects. As in the uterus, the β-ATP peak was shifted significantly relative to that of skeletal and cardiac muscle (0.4 ppm) in both relaxed and stimulated tissues. Calibrated solutions of the β-ATP shift relative to PCr as a function of Mg^{++}, pH, ATP, and ionic strength indicated that the chemical shift difference is most likely due to a low smooth muscle free Mg^{++} level (0.4 mM) relative to that of striated muscle (> 1 mM). Because of the similar biochemical and NMR characteristics of bladder and uterus, we conclude that these parameters reflect basic properties of visceral smooth muscle.
Supported by NIH (AM14485) and the Francis Bitter National Magnet Laboratory at MIT. PFD supported by NRSA (AM06506), RAM by the Juvenile Diabetes Foundation, and MJK by RCDA (AM00178).

T-AM-Pos28 SELECTIVE AND NON SELECTIVE SPIN LATTICE RELAXATION TECHNIQUES FOR MEASURING CHEMICAL EXCHANGE IN METABOLIC ^{31}PHOSPHATES WITH NMR. R. L. Coulson, Department of Physiology, J. D. Cutnell, Department of Physics, Joel Gober, Molecular Science Program, Southern Illinois University, Carbondale, Illinois 62901.

^{31}P Nuclear Magnetic Resonance in the Fourier Transform Mode provides opportunities to investigate chemical exchange rates among the energy compounds of significance in contracting muscles. To understand the energy metabolism of the contracting heart it is necessary to determine exchange rates between ATP and ADP mediated by myosin ATPase and PCr and ADP mediated by creatine phosphotransferase simultaneously in the living muscle. In inversion labeling experiments on model systems including all these metabolic factors chemical exchange among the phosphorus compounds can be detected and forward and reverse reaction rates can be estimated. Using these techniques it is apparently possible to determine the proximity to or remoteness from equilibrium for the reactions involved since both concentration and reaction rates can be obtained from NMR spectroscopy.

T-AM-Pos29 INTERFILAMENT FORCES IN VERTEBRATE STRIATED MUSCLE AT LONG SARCOMERE LENGTHS. T.C. Irving and R.M. Millman, Biophysics, Physics Dept., University of Guelph, Guelph, Ont. Canada, N1G 2W1.

Curves relating lattice spacing and applied pressure have been obtained for the A-band of chemically-skinned frog muscles stretched beyond filament overlap in relaxing and rigor solutions using an osmotic stress technique. Similar curves for frog sartorius muscle at short sarcomere lengths (Millman, 1981, J. Physiol. 320:118P) showed that the lattice spacing at zero applied pressure is greater in relaxed than in rigor muscle, spacings are the same at a pressure of about 20 torr, and the spacings are less in relaxed than in rigor muscle at higher pressures. Previously, the major part of the difference between the curves at low pressures was attributed to an attractive pressure exerted by actin-myosin crossbridges in rigor. At long sarcomere lengths, however, the actin filaments are pulled out from the A-band lattice, preventing the formation of crossbridges: hence the observed spacings should be free of any effects of such crossbridges. As expected from electrostatic theory, the spacings at long sarcomere lengths are considerably smaller than the corresponding spacings at shorter sarcomere lengths under both relaxed and rigor conditions. Both relaxed and rigor curves at long sarcomere lengths have shapes that are very similar to the corresponding curves at short sarcomere lengths; i.e. the spacing difference (relaxed minus rigor) is positive at zero applied pressure, negative at high pressures and zero at a pressure of about 20 torr. These results indicate that the observed differences in lattice spacing cannot be caused by actin-myosin crossbridges. The difference must therefore involve some or all of the following factors: long-range (e.g. electrostatic) forces, myosin-myosin links, or other structures which show differences between the relaxed and rigor states over a range of sarcomere lengths. Our current data tend to support the first (electrostatic) alternative.

T-AM-Pos30 LATERAL FORCES BETWEEN ACTIN FILAMENTS IN MUSCLE. B. M. Millman and R. M. Bell, Biophys. Interdepartmental Group, Physics Dept., University of Guelph, Guelph, Ont. Canada N1G 2W1

In several muscle preparations, a diffuse equatorial X-ray reflection is observed which has been associated with the actin filaments alone. In intact muscles, this reflection corresponds to a lattice spacing between 11.5 and 14.5 nm. We have used this reflection to study the lateral forces between actin filaments in several preparations: chemically-skinned anterior byssus retractor muscle of Mytilus, glycerol-extracted scallop striated adductor muscle, and chemically-skinned frog semitendinosus muscle at sarcomere lengths greater than 3.6 μm. In all cases, we have measured the spacing of the actin filament lattice as a function of applied external pressure generated by large polymeric molecules (dextran). As found in similar systems (e.g. TMV: Millman et al., 1982, Biophys. J. 37:379a) the actin spacing decreases with increasing applied pressure from a value of 14 - 19 nm at zero applied pressure to 8 - 10 nm at high pressures. The curve obtained lies close to the calculated curves for electrostatic pressure assuming hexagonal packing of the filaments, a filament charge of 15 electrons/nm and a charge diameter of 8 - 10 nm. Glycerol-extracted rabbit psoas muscle treated with 0.6 M KCL solution to dissolve the myosin filaments gives similar reflections from an actin-based lattice, but the spacings are about double those from the pure actin lattice. They lie close to the electrostatic pressure curve for a charge diameter of 20 nm. This larger lattice corresponds, we believe, to a lattice of actin filaments decorated with myosin molecules. The above data suggests that actin filaments on their own can form a close-packed lattice stabilized by a balance between electrostatic and van der Waals forces. Actin filaments decorated with myosin form a close-packed lattice but with a charge diameter of about 20 nm.

T-AM-Pos31 THICK FILAMENT DIAMETERS IN LONG AND SHORT LIMULUS MUSCLE. B.M. Millman and J.E. Hegney, Biophysics Interdepartmental Group, Physics Dept., Univ. of Guelph, Guelph, Ont., Canada, N1G 2W1.

Thick filaments from Limulus muscle appear to be unique in that they shorten when the muscle is contracted below the region where a conventional sliding filament mechanism operates. The mechanism involved in filament shortening remains a mystery, but it has been shown (Dewey et al, 1979, Cross-bridge Mechanisms in Muscle Contraction pp. 3-22) that thick filament shortening is accompanied by an increase in thick filament diameter. We have explored this latter phenomenon by obtaining X-ray diffraction measurements of lattice spacings in glycerol-extracted muscle subjected to externally-applied osmotic pressure by large polymeric molecules (dextran). The curve relating lattice spacing to applied pressure is quite sensitive to the filament diameter, and in cases where electrostatic forces dominate, can be used to calculate the effective filament diameter (Millman and Nickel, 1980, Biophys. J. 23:49). Limulus telson muscles were glycerol-extracted at either long or short lengths. Both groups of muscles were equilibrated in dextran solutions of various concentrations and the thick filament lattice spacings were determined by X-ray diffraction. The pressure/spacing curves for both long and short muscles show a characteristic decrease in spacing with increasing pressure. The curve from long muscles has low scatter in the data and lies close to the theoretical curve for electrostatic pressure using a thick filament diameter of 45 nm. The points from short muscles are more scattered but all lie at greater spacings than corresponding points from long muscles (an average of about 5 nm greater). These data strongly support a difference in thick filament charge diameter between long and short muscles of about 5 nm, consistent with a thickening of the filaments at short muscle lengths as observed by Dewey et al.

T-AM-Pos32 DOES LATENCY RELAXATION DEPEND ON EXTERNAL SERIES COMPLIANCE? Louis A. Mulieri and Norman R. Alpert, Dept. Physiol. & Biophys., University of Vermont, Burlington, Vermont 05045.

In previous experiments (Mulieri & Alpert, Biophys. J. 37 108a) to determine if the depth (R) of the latency relaxation (LR) depends on the number of sarcomeres in series, interpretation of the results were complicated by unintentional changes in amount of tendon in series with the sarcomeres. Hence experiments were performed (bundles of 20-40 fibers from frog's anterior tibialis M., massive stimulation at 1 min intervals at a sarcomere length of 3μm and 15°C) at a constant sarcomere length and number but with external series compliance varied by changing tendon length or by increasing lever compliance. To vary length of tendon, bundles (6-9mm fiber length x .07-.15mm^2 cross section) were prepared with one normal tendon (0.8-2mm x .05-.06mm^2) and one long tendon (5-13mm x .02-.05mm^2). The latter was connected to the isometric transducer lever so that 0.5mm, 6mm, or 13mm of its length was in series with the fiber. There was no change in R when tendon length was varied over this range (1-13mm) showing that amount of tendon does not play a role in determining R. Series compliance was also increased by replacing one isometric lever with a compliant glass lever having adjustable length. This reduced R approximately in proportion to compliance (50% reduction occurring with 0.4±0.2μm/mg added compliance). Similar results were seen when compliance was increased by adding an inexcitable bundle in series. Paradoxically, time to beginning of positive twitch tension tended to decrease when R diminished. These results are consistent with the LR process behaving as an ideal force generator under physiological conditions. However with very large added compliance another physical model may be appropriate. U.S.P.H.S. 28001-01/P1.

T-AM-Pos33 MYOSIN P-LIGHT CHAIN PHOSPHORYLATION IN RAT SLOW-TWITCH MUSCLE. R.L. Moore and J.T. Stull, Dept. Pharmacology, University of Texas Health Science Center, Dallas, TX 75235.

Previous reports indicate that myosin P-light chain phosphorylation in fast-twitch rat skeletal muscle is correlated to isometric twitch potentiation (post-tetanic and staircase potentiation) in a curvilinear fashion. Increases in isometric twitch tension were associated with P-light chain phosphorylation when phosphate incorporation was equal to or exceeded 0.40 mol phosphate per mol P-light chain. Phosphorylation of P-light chain in slow-twitch skeletal muscle may be different since slow-twitch muscles exhibit no potentiation of isometric twitch tension.

The phosphate content of P-light chain in rat soleus muscle in situ was 0.00 mol phosphate per mol P-light chain when the muscle was quiescent or repetitively stimulated to produce isometric contractions via the sciatic nerve at 1 Hz and 5 Hz at 37°C. Stimulation of the muscle at 30 Hz and 100 Hz resulted in phosphorylation to maximal values of 0.20 ± 0.06 and 0.33 ± 0.06 mol phosphate/mol P-light chain, respectively. No potentiation of the isometric twitch was observed under these conditions. The rate of dephosphorylation of P-light chain in rat soleus muscle was 0.027 s^{-1} which was significantly faster than the rate of dephosphorylation found in rat fast-twitch muscle (0.007 s^{-1}). Myosin light chain kinase activity was 16 ± 3 and 54 ± 1 nmol phosphate incorporated/min·mg protein in extracts from rat slow-twitch soleus and fast-twitch white gastrocnemius muscles, respectively.

These results suggest that the smaller amount of P-light chain phosphorylation in repetitively stimulated rat soleus may be due, in part, to quantitative differences in myosin light chain kinase and phosphatase activities. The lack of potentiation of isometric twitch tension may be related to an inability to incorporate at least 0.4 mol phosphate per mol P-light chain.

T-AM-Pos34 POTASSIUM DEPOLARIZATION AND PHENYLEPHRINE PRODUCE DIFFERENT AEQUORIN SIGNALS IN VASCULAR SMOOTH MUSCLE. Kathleen G. Morgan and James P. Morgan, Depts. of Pharmacology and Medicine, Mayo Foundation, Rochester, MN 55905.

Aequorin, which emits light in the presence of Ca^{++}, was loaded into smooth muscle cells of the ferret portal vein by rendering them temporarily hyperpermeable with EGTA (Morgan & Morgan, Pflügers Archiv., in press 1982). The addition of phenylephrine (PE) to the bathing solution or the replacement of equimolar amounts of NaCl with KCl produces similar tonic contractions of the muscle. In contrast, the light (Ca_i) responses elicited by the two stimuli are qualitatively different. K depolarization generally produces a simple sustained rise in light whereas PE produces an initial spike of light (duration at 1/2 amplitude: 20-100 sec) which rapidly falls to a more slowly falling plateau. PE is thought to release Ca^{++} from intracellular stores and the initial spike may reflect local Ca^{++} levels which are transiently very high only in the vicinity of the storage site, although this seems unlikely over the time courses involved. The plateau levels of light (over the course of 3-15 minutes) are even less likely to reflect Ca^{++} inhomogeneities within the cell. For equal levels of tension, the level of the light plateau is much lower in the presence of PE than with K depolarization. If cellular Ca^{++} heterogeneities can be ruled out, the lower light/tension ratio in the presence of PE must be due either to an increased sensitivity of the contractile apparatus to Ca^{++} in the presence of PE; or, to the existence of Ca^{++}-independent tension during the steady state. The second possibility seems unlikely since when Ca_i^{++} levels are decreased in the continued presence of PE (by decreasing extracellular Ca^{++} to $< 10^{-6}$M), tension falls. Support: HL 27847 and HL 12186.

T-AM-Pos35 INTRACELLULAR CALCIUM TRANSIENTS DURING ACTIVE SHORTENING OF MAMMALIAN CARDIAC MUSCLE. P.R. Housmans, N.K.M. Lee, and J.R. Blinks, Dept. of Pharmacology, Mayo Fdn., Rochester, MN 55905.

In many kinds of striated muscle the terminal (isometric) phase of relaxation occurs earlier when the muscle is allowed to shorten during a twitch than when it is constrained to contract isometrically throughout. We sought an explanation for this so-called shortening deactivation in terms of changes in the intracellular calcium transients of cat papillary muscles detected with the Ca^{++}-sensitive bioluminescent protein aequorin. Aequorin (light) signals, force, length, and shortening velocity were recorded in contractions in which loads were manipulated with a servo-controlled electromagnetic lever to control the extent and timing of shortening. Light intensity was always greater when active shortening was taking place than under comparable conditions when it was not. The extra light (light of shortening) was distinguishable within 30 msec of the onset of shortening and its intensity roughly paralleled the velocity of shortening. The observed changes in the aequorin signals were larger than could be attributed to the influence of length per se (Allen & Kurihara, J. Physiol. 327:79, 1982), and we have ruled out the possibility that our light records are subject to motion artifacts of that magnitude. We conclude that the act of shortening (or the absence of tension development) slows the decline of cytoplasmic [Ca^{++}] whenever it occurs during the calcium transient. A possible explanation for the apparently paradoxical association of increased myoplasmic [Ca^{++}] with shortening deactivation is that shortening decreases the number of attached cross-bridges. This in turn may decrease the affinity of troponin C for Ca^{++} (see Weber & Murray, Physiol. Rev. 53:p.655, 1973). Support: USPHS Grant HL 12186 and International Research Fellowship TW 03046.

T-AM-Pos36 COHERENT IMAGING OF MOLECULAR MOTIONS IN ACTIVATED FIBRES OF SKELETAL MUSCLE Mark Sharnoff and Lawrence P. Brehm, Department of Physics, University of Delaware, Newark, DE 19711

During the last several years we have used a very sensitive differential holographic method to image the changes which occur in the visual appearance of muscle fibres undergoing isometric contraction[#]. Because the method is by nature interferometric, gross displacements of portions of a fibre, such as might occur during passive stretch, are imaged as readily as are the fine, submicroscopic motions associated with active contraction; an unambiguous and impeccable discrimination between these two contributions to intensity in our differential images has heretofore not been possible. We have modified our technique by employing exposure flashes unequal in length or object path length shifts which differ slightly from ½ wavelength. While these modifications spoil the degree to which the static components of an object are suppressed in our differential images, they do permit the changes in the object wave to interfere with the residual portion of the static parts of the object wave. Changes in the object wave's amplitude can then be discriminated from changes in its phase. By obliquely illuminating the object so that any gross displacement is translated into changes in the phase of the object wave, it becomes possible also to distinguish between gross displacement and local, submicroscopic motion. In this way we have shown that the myofibrillar patterns which occur in our differential images of the onset of contraction are principally the result of the latter.[@]

[#]M. Sharnoff and H. A. Kunkel, III, Biophys. J. 25, 140a (1979)

[@]M. Sharnoff and L. P. Brehm, this meeting (accompanying abstract)

T-AM-Pos37 THE UNIT OF ACTIVATION IN SKELETAL MUSCLE IS MYOFIBRILLAR, NOT SARCOMERIC.
Mark Sharnoff and Lawrence P. Brehm, Department of Physics, University of Delaware, Newark, DE 19711

With the help of the coherent imaging method described in an accompanying abstract, we have studied the onset of contraction in single fibres of frog semitendinosus muscle excited by individual global supra-threshold transverse current pulses. We find that sarcomeres are not activated at random. Rather, within any myofibril, activation occurs in units typically of 20 to 30 contiguous sarcomeres which are uniformly and, to resolution of 0.2 msec, simultaneously recruited. Recruitment is accompanied by an immediate, prominent, and long-lasting reduction of the light coherently scattered by the unit. The reduction in scattering amplitude is about 30%; its substantial size indicates that it occurs primarily within the myofibrils themselves rather than in optically unresolved portions of the sarcoplasmic reticulum which lie adjacent. While the myofibrils initially activated appear to be randomly located, the distribution of activation at any point in the development of tension closely repeats from contraction to contraction. It is characteristically monomyofibrillar. Although groups of adjacent myofibrils may occasionally become activated together, we find no evidence that regularity of sarcomeric registration within such groups is essential for their occurrence. The activated myofibrils are too numerous to be associated with nuclei or with clusters of mitochondria. Over the range 2.0 μm $\leq\sigma\leq$ 3.5 μm the results we have sketched show no strong dependence upon striation spacing.

T-AM-Pos38 Perinatal Changes in Contractility of Rat Myocardium. Zia J. Penefsky Dept. of Physiology & Biophysics, Mt. Sinai Sch. of Medicine, New York.

Mechanical responses and calcium sensitivity were studied in hearts from fetal rat at term, and during the first four postnatal weeks. At term, fetal rat heart exhibits a positive force-frequency (F-f) response and high calcium sensitivity, similar to other mammalian species. Within the first postnatal week, the positive F-f response is reversed and calcium sensitivity is diminished. This process is progressive during the second and part of the third postnatal week.By the end of of the fourth postnatal week, the F-f response has reversed to an initial positive followed by a negative pattern as the frequency of stimuli is increased. Greater than normal concentrations of calcium (2.7 mM) are no longer inotropic.

Recovery from stress also reflects developmental changes. In the fetal heart, a potentiated post-rest response monophasically declines to a steady-state. After the first postnatal week, post-rest recovery is sub-normal. The response increases subsequently and there is a rebound before the response declines to a steady-state. At four weeks of age, recovery is much quicker, with no appreciable rebound. These events may be explained in terms of specufic developmental changes in ionic currents across the sarcolemmal membrane of the heart.

T-AM-Pos39 EFFECT OF GLUCOCORTICOIDS ON Ca SENSITIVITY IN RAT FAST-TWITCH AND SLOW-TWITCH MUSCLE FIBERS. Barbara Laszewski, Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195.

Pharmacological doses of glucocorticoids induce skeletal muscle weakness and atrophy with a preferential effect on fast-twitch muscle. The possibility that decreased myofibrillar Ca sensitivity caused the weakness was investigated using the skinned fiber technique. Following treatment with either 1.5mg/kg/day dexamethasone or saline for 14 days (IM), rat extensor digitorum longus (EDL)(fast-twitch) and soleus (slow-twitch) muscles were excised and chemically skinned. Single muscle fibers were isolated and tension was recorded over a pCa range of 7.0 to 4.0. The EDL pCa/tension relationship was not altered by glucocorticoid treatment, however, the soleus fibers from dexamethasone-treated animals had slightly decreased Ca sensitivity. Since the twitch and tetanic force produced by soleus muscles was not reduced by glucocorticoid treatment, the reduced Ca sensitivity in soleus fibers is probably not physiologically important. Therefore, decreased Ca sensitivity does not appear to be the mechanism of the selective fast-twitch fiber muscle weakness induced by glucocorticoid treatment. Supported by NIH grants NS00498, NS16696.

T-AM-Pos40 FORCE-pCa HYSTERESIS AT VARIOUS FREE Mg^{+2} CONCENTRATIONS IN SKINNED BARNACLE MUSCLE. D.A. Martyn and A.M. Gordon, University of Washington, Seattle, Washington 98195.

Bundles of myofilaments were isolated by mechanical dissection of single muscle fibers from the giant barnacle, *Balanus nubilus*. The isometric force-pCa relation was measured by immersing the filament bundles into baths of varying pCa and free Mg^{+2} concentration. Solutions contained Na propionate (30mM), K propionate (170mM), EGTA [ethylenebis (oxyethylene-nitrilo) tetraacidic acid] (30 mM), MgATP (4.5mM) and pH 7.1. Internal membranes were destroyed by treatment with Triton X-100. Force-pCa relations were determined in two ways: 1) by stepping up in $[Ca^{+2}]$ to a submaximal and then to a maximal $[Ca^{+2}]$ and 2) by stepping from maximal $[Ca^{+2}]$ down to the submaximal level. In all experiments, the steady force-pCa relation shifted toward greater sensitivity when determined by method 2, indicating a hysteresis in the force-pCa relation. Hysteresis was also evident when the fiber was stepped from a low $[Ca^{+2}]$ to a higher, but submaximal $[Ca^{+2}]$, and back to a low $[Ca^{+2}]$. The degree of hysteresis increased as the intervening $[Ca^{+2}]$ or force increased toward maximal. Force-pCa hysteresis was characterized with free $[Mg^{+}]$ of 0.5, 1.5, and 5.5mM. Decreasing free $[Mg^{+2}]$ shifted the force-pCa relation determined by both methods toward greater $[Ca^{+2}]$ sensitivity. Experiments were performed where pCa was held constant at a submaximal level and the fiber exposed first to 5.5mM (low sensitivity) then 0.5mM (high sensitivity) and back to 5.5mM $[Mg^{+2}]$. With constant pCa the fiber developed more force on the second than first exposure to 5.5mM free Mg^{+2}, again showing hysteresis. The results suggest that the hysteresis might not be related to $[Ca^{+2}]$, per se, but may depend on the intervening force. Supported by NIH NS 08384.

T-AM-Pos41 EQUATORIAL X-RAY DIFFRACTION FROM SINGLE SKINNED RABBIT PSOAS FIBERS DURING VARIOUS DEGREES OF ACTIVATION. B. Brenner and L. C. Yu. NIADDK, NIH, Bethesda, MD, 20205.

Single skinned rabbit psoas fibers were used to record equatorial X-ray diffraction patterns during isometric contraction. This preparation avoids the problems of limited ATP diffusion which complicates activation in multiple fiber preparations. However, even in single skinned fibers the striation pattern tends to become disordered within seconds of isometric activation. Recently a technique was developed where fibers were cycled between isometric steady state and unloaded isotonic shortening (Brenner, Biophys. J., 1983). With this technique the striation pattern remains well ordered for up to 2-3 hours. During this time force and speed of shortening decrease only up to 10-20%. We used this technique to record equatorial X-ray diffraction patterns during the period of isometric contraction with force levels ranged between 8 and 100% of full Ca^{++} activation (5°C, μ = 170 mM). Fibers were constantly monitored by light microscopy and laser diffraction for integrity and homogeneity of the striation pattern. Preliminary results showed that the intensity ratio of the first two reflections (I_{11}/I_{10}) increased in an almost linear way with force, similar to results obtained from frog sartorius (Yu et al., JMB, 1979). The lattice spacing decreased from 438Å with increasing force, plateauing off at 380Å when force reached approximately 80% of the maximum value. It is interesting to note that this plateau level is identical with the plateau level reached in relaxed muscle when ionic strength is lowered to $\mu \leq 50$ mM (Brenner et al. this meeting). This supports the idea that the spacing changes observed in relaxed muscle when lowering ionic strength may be due to the attachment of bridges (Brenner et al., PNAS, 1982) and suggests that the radial forces produced by bridges attached in a relaxed muscle at low ionic strength are of the same order of magnitude as the radial forces produced by bridges attached during contraction.

T-AM-Pos42 THE RELATIONSHIP BETWEEN FORCE AND VELOCITY OF SARCOMERE SHORTENING MEASURED BY WHITE LIGHT DIFFRACTION. Y.E. Goldman, Dept. of Physiol., Univ. of Penna., Phila., PA 19104

A diffraction instrument to measure muscle fiber striation spacing has been constructed using polychromatic light scattering to avoid Bragg-angle artifacts (Rüdel & Zite-Ferenczy, J. Physiol.290,317,1979). Collimated light from a Xenon arc lamp is first scattered by an Acousto-Optic (A-O) light beam deflector acting as a variable-spacing diffraction grating and is then focused on to the muscle fiber so that the meridional incident angle varies with wavelength. A second diffraction by the cross striations reverses the chromatic dispersion. The reciprocal of the frequency of the A-O driving signal (≅50MHz) is proportional to average sarcomere length. During active and passive shortening of frog skinned muscle fibers, the sarcomere length, recorded with white light diffraction, decreases smoothly without pauses. V_{max} measured by the slack test method (Edman, J. Physiol.291,143,1979) is markedly higher than that estimated by extrapolation of the force-velocity hyperbola as previously reported by Julian and Moss (J. Physiol.311,179,1981). Skinned fibers shortened at over 4μm/s per half sarcomere (Rana temporaria, semitendinosus; 5°C; ionic strength, 0.2M). However, isotonic contractions at very low loads (P/P_o<5%) indicate a deviation of the force-velocity curve from a hyperbola toward higher velocities. Polymers (PVP-40, 40-60g/l; Dextran T-500, 50g/l) reduce shortening velocity at low loads. Since these polymers also shrink lateral filament spacing (Goldman, Matsubara & Simmons, J. Physiol. 295,80P,1979) and increase skinned muscle fiber stiffness (Goldman & Simmons, Biophys. J.21,86a,1978), the present results suggest that the shape of the cross-bridge force-extension curve affects V_{max} as expected from Huxley's (Prog. Biophys.7,255,1957) theory. Supported by NIH grants AM00745 & AM26846.

T-AM-Pos43 THERMOELASTICITY IN RIGOR MUSCLE. Susan H. Gilbert, Anat. Sci., SUNY at Stony Brook, Stony Brook, NY 11794, and Lincoln E. Ford, Dept. Med., U. Chicago, Chicago, ILL 60637.

Rigor muscle was studied to assess the thermoelasticity of the contractile apparatus in the absence of crossbridge enthalpy changes. Thermal changes during stretch and release were expected from two sources: thermoelasticity (expected to produce thermal changes of opposite sign for stretches and releases) and frictional or viscous resistance to movement (expected to produce heat liberation with both stretches and releases). Rigor was induced by placing pairs of frog sartorius muscles in physiological solution containing 0.4 mM iodoacetate and 1.5 mM NaN_3 at 0C overnight. Length changes of 0.1-0.55 mm complete within 1-15 msec were applied to the tendon end of the muscles. Heat was measured with a thermopile between the muscles. Force was always measured at the tendon end and sometimes at the pelvic end as well. A difference in force between the two ends of the muscle indicated a resistance to movement over the thermopile. Stress relaxation at the tendon end was accompanied by an inverse force change at the pelvic end and indicated a viscous resistance between the muscle and the thermopile. Muscles which showed little stress relaxation always showed cooling with stretch. In these muscles the heat:tension ratios ranged from -0.007 to -0.015 $(mJ/g)/(kN/m^2)$ for stretches and releases and were always greater in releases than in stretches. The differences were greater in preparations showing more stress relaxation, indicating that heat from movement contributed to the thermal changes measured. The thermoelastic coefficient is likely to be between the values for stretch and release because correction for heat from movement will decrease the heating ratio for releases and increase the cooling ratio for stretches. Supported by grants from NSF (PCM8120276) and MDA to SHG.

T-AM-Pos44 MYOSIN PHOSPHORYLATION DECREASES THE ATPASE OF CARDIAC MYOFIBRILS. K. Franks and R. Cooke, Dept. of Biochemistry/Biophysics and CVRI, University of California, San Francisco, CA 94143 and J.T. Stull, Department of Pharmacology, University of Texas, Dallas, TX 75235.

Our previous work has shown that myosin phosphorylation decreases the ATPase activity of lightly fixed skeletal myofibrils. We have now extended these results to myofibrils prepared from whole hearts of rabbits and rats. Myofibrils were phosphorylated by incubation in myosin light chain kinase, CaM and either ATP or ATPγS, for 15' at 20°C. The level of myosin regulatory light chain phosphorylation was 50-70% as measured using gel electrophoresis in the presence of urea. The ATPase activity of unfixed myofibrils was not changed by phosphorylation. The ATPase activity of unphosphorylated myofibrils was not altered by reaction with 0.01% glutaraldehyde for 5' at 0°C. Phosphorylation decreased the ATPase activity of fixed myofibrils. The measured activities (given in seconds $^{-1}$ per head) were for rat heart: control 1.8, phosphorylated 1.0; and for rabbit heart: control 1.1, phosphorylated 0.6. Thus the phosphorylation of cardiac myosin appears to have an effect that is similar to that found for skeletal myofibrils and fibers. Preliminary results using the slack test method to measure the maximum velocity of shortening (Vm) in glycerinated skeletal fibers at 35°C has found that thiophosphorylation decrease Vm from ∿6 lengths/sec to ∿4 lengths/sec. If one of the three components required for phosphorylation is omitted there is little change in either phosphorylation or Vm. These results suggest that in both skeletal and cardiac muscle, myosin phosphorylation decreases the rate of cross-bridge cycling resulting in both decreased energy expenditure and decreased velocity of shortening. Supported by Grants from USPHS to R.C. AM00479 and HL16683 and to J.T.S. HL23990.

T-AM-Pos45 MYOSIN ISOENZYMES AND THE VELOCITY OF PAPILLARY MUSCLE SHORTENING. Pagani ED, Gonzalez M, and Julian FJ. Dept.Anes.Res.Labs., Brigham and Women's Hosp., Boston, MA

The aim of the experiments reported here was to determine if the unloaded velocity of papillary muscle (PM) shortening is related to the relative proportions of myosin isoenzymes. The adult rabbit ventricle (RV) contains 3 isoenzymic forms of myosin, denoted RV_1, RV_2, and RV_3 according to their mobility on pyrophosphate gels and relative Ca-ATPase activity ($RV_1 > RV_2 > RV_3$). In addition, the relative proportions of myosin isoenzymes change in response to thyroxine (T_4) administration (Martin, Pagani and Solaro, Circ. Res. 50:1982). To test our hypothesis, we injected rabbits ages 4 wks to 6 mos with saline or T_4 (150 µg/kg) for 7 days. A suitable PM was dissected from the right ventricle, mounted in an experimental chamber, and the unloaded velocity of shortening was determined as described by Julian et al. (Circ. Res. 49:1981). The same PM was also evaluated for the distribution of myosin isoenzymes essentially as described by Martin et al. (ibid). We found that the PM of 4 wk old rabbits contained almost 100% RV_1 myosin. Around 5 wks of age the RV_3 myosin appeared at which time the percentage of RV_1 began to decline reaching about 30% of the myosin population by 3 mos. By 6 mos of age, the PMs contained about 10% RV_1 myosin. Irrespective of initial age, after 7 days of T_4 administration the PMs contained essentially all RV_1 myosin. Moreover, we observed a high correlation ($r = 0.96$) between the unloaded velocity of shortening and the percentage of RV_1 myosin. PMs that contained 5% RV_1 myosin shortened at a velocity of 0.4 muscle lengths/sec at 30°C whereas PMs that contained 100% RV_1 myosin shortened at a velocity of 3.8 muscle lengths/sec under the same conditions. We conclude that the unloaded velocity of papillary muscle shortening is dependent, at least in part, on the relative proportions of myosin isoenzymes. Supported by USPHS HL 30133 (FJJ) and MDA (EDP).

T-AM-Pos46 UNIFORM SARCOMERE BEHAVIOUR DURING MYOCARDIAL CONTRACTION IN SINGLE CARDIAC CELLS. N.M.De Clerck,V.A.Claes and D.L.Brutsaert,University of Antwerp,Antwerp, Belgium.

Sarcomeres of intact, single cardiac cells were visualized through the objective of an inverted microscope. Striations were projected on a television screen. Sarcomere length was measured by analysing the frequency content, corresponding to the appearance of white and black striations in a selected television line. Distribution of sarcomere length in different areas of the resting cardiac cell proved to be uniform. Twitch contractions were initiated by electrical stimulation in a perfusion medium containing 1.8mM calcium. The entire contraction-relaxation cycle of the cell was recorded on videotape. From the tape, distribution of sarcomere length in different regions of the cell was analysed on stop frames at 20ms intervals. The cell and different groups of sarcomeres contracted simultaneously. Sarcomere length in different regions of the cell also behaved synchronously throughout contraction and relaxation. These results present arguments for uniformity of myocardial contraction in the intact single cardiac cell both in time and space.

T-AM-Pos47 RIGOR-RELAXED TRANSITION IN SKINNED SKELETAL MUSCLE FIBERS: DIRECT MEASUREMENT OF OPTICAL DEPOLARIZATION CHANGES. Y. Yeh, R. J. Baskin, M. E. Corcoran and R. L. Lieber, Departments of Applied Science and Zoology, University of California, Davis, Calif. 95616. Optical polarization properties of light diffraction spectra from single or small bundles of skinned striated muscle fibers exhibit large changes when chemically treated to effect the rigor state. This technique, which combines optical diffraction and ellipsometry measurements, had previously been shown by Pinsky and Yeh[1] to be a sensitive probe of periodic anisotropic regions of the fiber, shows marked decrease in the measured phase change phase angle, δ, as the fiber approaches the rigor state. The degree of phase angle change is a function of sarcomere length: Maximum overlap ~ 2.5 μm gave the most change in δ. (~ 40° decrease for a bundle of three fibers.) At a sarcomere length of 2.9 μm this δ-value changes by ~ 50% the previous value. At a non-overlapping length of ~ 3.8 μm, δ does not vary at all upon the removal of ATP. The rigor state was confirmed by stiffness measurements made after microscopic (1%) quick length changes. Upon re-relaxation, the stiffness of the skinned fiber decreases to the value of the resting state (4 mM ATP) and the phase δ returns to its original value. A model based on anisotropic S-2 movements is suggested. (Work supported by the NIH under Grant # AM 26817.)

1. Pinsky, B. G. and Yeh, Y., Biophys. J. 37, 360a, (1982), Yeh, Y. and Pinsky, B. G. "Optical Polarization Properties of the Diffraction Spectra from Single Fibers of Skeletal Muscle" (submitted to Biophys. J. (1982).

T-AM-Pos48 FRAUNHOFER DIFFRACTION OF LIGHT BY STRIATED MUSCLE: THE MYOFIBRIL AS A PHASE GRATING. J.P. Payne, M.M. Judy, G.H. Templeton, and T.D. Black*. University of Texas Health Science Center at Dallas, Department of Physiology, Dallas, TX 75235 and *University of Texas at Arlington, Department of Physics, Arlington, TX 76019.

Monitoring sarcomere length in a contracting striated muscle fiber using a laser probe, though a valuable technique, can be significantly affected by complex effects such as 3-D Z-disk misregistry and multiple diffraction (Yeh, Y. et. al., Biophys J 29:509-522, 1980). Perhaps deeper insight into the contraction cycle may be gained by using isolated myofibrils, which are simpler optical systems and may reveal subtle mechanical events masked in an averaged tension response produced by many myofibrils in a single fiber (Borejdo, J., J Mechanochem Cell Motil 4:189-204, 1977). Therefore, we have derived analytical expressions for far-field (Fraunhofer) diffraction spectra produced by a single myofibril in which the sarcomere is taken to be the fundamental repeat unit. As a single myofibril obeys the Raman-Nath limit, the problem of multiple diffraction is eliminated and the diffracted field is given by the 2-D Fourier transform of the linear sarcomere array and the transmission function of a single sarcomere. In general, the transmission function describes how an optical element changes the phase and amplitude of the incident light, but, by considering the fibril to be transparent, the amplitude remains unchanged. Thus, the myofibril behaves as a phase grating. We have examined the effects of sarcomere length, fibrillar diameter, optical birefringence, and the contributions due to the H zone, M-line, Z-disk, I band, and the overlap region, whose indicies of refraction are related to local protein concentration, on computer-generated diffraction patterns in order to assess the sensitivity of such spectra to detect structural changes.

T-AM-Pos49 LIGHT DIFFRACTION MICROSTRUCTURES OF SINGLE ACTIVATED MYOCARDIAL CELLS. Alfred F. Leung. Department of Physics, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

High resolution laser diffraction spectra of striated muscle were taken with an argon-ion laser at different wavelengths. The positions of the microstructures lying within the diffraction columns were measured. The spectral shifts of the microstructures due to a change in the wavelength of the laser beam followed the grating equation. Each microstructure was then interpreted as the diffraction from a group of sarcomeres of nearly identical length. The movements of the microstructures of single unattached myocardial cells during phasic contractions were recorded. After the cell was stimulated electrically, each microstructure representing the diffraction from a group of sarcomeres remained stationary for about 15 ms, then moved away rapidly at a speed of about 8 μms^{-1} per sarcomere, and returned to its original position. The period of the contraction-relaxation cycle was about 1.2 s. The movements of the microstructures indicated that the sarcomeres shortened smoothly, uniformly and independently. The latent period was taken to be the time between the electrical stimulus and the onset of movement of the microstructure. Measurements showed that the latent period was dependent on the free $[Ca^{2+}]$ of the bathing solution of the cell. The observed decrease in the microstructure width as the cell shortened was explained by the proportionality between the sarcomere length and sarcomere length dispersion. The intensity of the microstructure during sarcomere shortening followed the prediction of the grating model. However, the absolute intensity maximum near the midpoint of the relaxation of the cell could not be explained by any intensity calculation which involves only dimensional or length parameters.

T-AM-Pos50 INVOLVEMENT OF CALMODULIN IN TENSION DEVELOPMENT BY SKINNED MUSCLE FIBERS OF THE RABBIT AT LOW LEVELS OF ACTIVATING CA^{2+}. R.L. Moss* and M.L. Greaser+, Dept. of Physiology* and The Muscle Biology Lab.+, Univ. of Wis., Madison 53706 (Intr. by J. Anderegg).

This study was undertaken to investigate further the form and basis of the tension-pCa relation in mammalian fast-twitch skeletal muscle. Single fibers were obtained from rabbit psoas muscles and were skinned in relaxing solution containing 5 mM EGTA. Steady isometric tension (P) was measured at 15°C as a function of pCa in the range 6.86-5.49. The Hill plot of tension-pCa data obtained at a sarcomere length of 2.46-2.62 μm was well-fit by two straight lines interesecting near pCa 6.50. For pCa < 6.50, the Hill coefficient (n) was 1.99; for pCa > 6.50, n was 6.67. Many of the fiber segments were treated with a solution containing 50 mM KCl, 20 mM EDTA, 5 mM PO_4 buffer, pH 7.0 at 30-34° for 120', a protocol which is known to partially extract TnC and myosin LC_2 (Moss, et al., JBC, 257:8588, 1982). The Hill plot of tension-pCa data obtained following this treatment was markedly different from the control plots in that the data was well-fit by a single straight line (n= 2.17). However, readdition of TnC and/or LC_2 to the EDTA treated segments was without further effect on the Hill plot, i.e., the Hill plot was still well fit by a single straight line. Subsequent addition of purified calmodulin to the treated segments resulted in a substantial reversal of the Hill plot to its original biphasic form. Since calmodulin is the Ca^{2+}-binding regulatory subunitof myosin light chain kinase (MLK), these results suggest the possibility that phosphorylation of LC_2 may somehow be involved in the process of tension development, at least at low levels of activating Ca^{2+}. (Supported by grants from NIH; the MDA and the American Heart Assoc.)

T-AM-Pos51 V_{max} MEASUREMENTS IN MAXIMALLY Ca^{2+}-ACTIVATED SINGLE SKINNED MUSCLE FIBERS AT LOW LEVELS OF TENSION PRODUCED BY THE PARTIAL EXTRACTION OF TnC. Marion L. Greaser+ and Richard L. Moss*, Dept. of Physiology* and The Muscle Biology Lab.+, University of Wis., Madison, WI 53706.

The mechanism of the reported effect of Ca^{2+} upon mechanical V_{max} (eg., Julian and Moss, J. Physiol., 311:179, 1981) remains unclear. The present study was designed to test whether the reductions in V_{max} at low Ca^{2+} are the result of an internal load as suggested by Thames, et al. (J. Gen. Physiol., 63:509, 1974). Such an internal load would retard muscle shortening, an effect that would be most noticeable at low Ca^{2+} due to a reduced number of attached cross-bridges. Single fibers were obtained from rabbit psoas muscles and were chemically skinned in a relaxing solution containing 5 mM EGTA. Maximum isometric tension and V_{max} were measured at 15°C and pCa 5.49 in untreated control fiber segments, in the same segments following extraction of TnC and, finally, following recombination of TnC into the segments. The extraction was done at 15°C in 20 mM EDTA buffer (Moss, et al., JBC, 257:8588, 1982). Since this solution also extracted LC_2, it was necessary to recombine LC_2 into the segments immediately following the extraction procedure. The tensions developed by the fiber segments following extraction and LC_2 recombination were as low as 30% of control values, indicating that a substantial loss of TnC occurred during extraction. At the same time, V_{max} was found to agree within 8% of the control value in the same segment (n=6). TnC recombination into the segments resulted in recovery of tension values to near control levels. Thus, at low levels of tension produced by partial extraction of TnC, and not by varying the $[Ca^{2+}]$, V_{max} remained at the high level typical of the untreated fibers. These findings indicate that decreases in tension per se, as seen when $[Ca^{2+}]$ is reduced, cannot account for reductions in V_{max}. (Supported by grants from NIH, the MDA and the American Heart Assoc.).

T-AM-Pos52 ANALYSIS OF INTENSITY OSCILLATIONS IN LIGHT DIFFRACTED BY SINGLE STRIATED MUSCLE FIBER. A.F. Leung and J.C. Hwang, Department of Physics, The Chinese University of Hong Kong and Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong.

Distinctive intensity oscillations in the diffraction lines were observed when a laser beam was incident normal to certain regions of isolated single muscle fibers of frogs. The intensity oscillations followed the first-order Bessel function suggesting the myofibrils to consist of cylinders. Such intensity oscillations could be due to the superposition of diffractions from individual myofibrils provided that the myofibrils in the illuminated regions were fairly uniform in diameters. The position of the intensity minima would then yield the average myofibrillar diameter. Starting from an area of the single muscle fibre which showed distinctive intensity oscillations, the beam of light was moved along the length of the fiber in small steps. The intensity oscillations decayed gradually with distance until they disappeared monotonically at some distance away from the distinctive area. Such gradual decay in the intensity oscillations could be due to a slow change in the myofibrillar diameter dispersion along the length of the fiber. Diffraction equations showed that when the diameter dispersion reached about 22%, diffraction line would decay monotonically. Electron micrographs of several fibres showed that there were about 25% diameter dispersion in most areas of the fibre. Such large dispersion would explain the low incidence of occurrence of distinctive intensity oscillations in most areas of single muscle fibres studied with laser diffraction techniques. (Supported in part by Wu Chung Research Fund)

T-AM-Pos54 ISOMETRIC TENSION MEASUREMENT IN SINGLE CARDIAC CELLS DURING ULTRAFAST CHANGES OF EXTRACELLULAR CALCIUM. Neal Shepherd (Introd. by E.A. Johnson), Downstate Medical Center, SUNY, Brooklyn New York 11203. A transducer has been developed to measure force isometrically from single bullfrog atrial cells. Motion of cell-bearing probes is monitored by phototransistors in the microscope ocular, the output voltage being proportional to force up to at least 1.2 uN. Probe stiffnesses vary from 1 to 5 uN/um with a natural frequency exceeding 1 kHz. Detector output is $>$ 200 mv/uN and noise (below 600 Hz) is equivalent to 5 to 10 nN depending on perfusion velocity. Cells were perfused transversely at rates up to 2.5 cm/sec making possible a change of ionic concentration at the level of the cell in less than 20 msec. at a time specifiable to $\pm$ 10 msec. For cells driven electrically at 30/min in 2 mM calcium, peak contraction force, F_p, was typically 250 nN with a time to peak, t_p, of 160 to 400 msec. Following a change of $(Ca)_o$ more than 200 msec. prior to a stimulus, F_p changed in the first beat to a new steady level proportional to $(Ca)_o^2$ for $(Ca)_o < 2$mM. Any change of $(Ca)_o$ within $\pm$ 200 msec of a stimulus markedly altered both F_p and t_p with a half-time of 100 msec. These data suggest minimal roles for extra- or intracellular calcium stores in e-c coupling in this tissue. When $(Ca)_o$ was reduced after the peak of a twitch, relaxation was accelerated. Increasing $(Ca)_o$ during that period had no measurable effect on tension time course. Aging cells developed $(Ca)_o$- sensitive tonic tension which rapidly declined upon reduction of $(Ca)_o$ but increased slowly upon return to high $(Ca)_o$. Asymmetric responses to $(Ca)_o$ in polarized cells may reflect rate-limiting steps in calcium extrusion not directly related to $(Ca)_i$.

T-AM-Pos55 ACTIVE CROSS-BRIDGE MOTION OF ISOLATED LIMULUS THICK MYOFILAMENTS AS REVEALED BY QUASI-ELASTIC LIGHT-SCATTERING STUDIES. Shih-fang Fan*, M.M. Dewey, D. Colflesh, Dept. of Anatomical Sciences and B. Chu, and K. Kubota, Dept. of Chemistry, SUNY at Stony Brook, NY 11794. *Shanghai Institute of Physiology, Academia Sinica, Shanghai, China.

Photoelectron count autocorrelation function of light scattered by Limulus thick myofilament suspensions was measured as a function of scattering angle at 25°C. By using the cumulants method of data analysis, the average line width $\bar{\Gamma}$ up to KL=120 was measured with K and L being the magnitude of the momentum transfer vector and the length of the filament, respectively. Ca^{++} (1.0-5.0 mM), besides inducing the shortening of the thick filament, caused a dramatic reversible increase in the $\bar{\Gamma}$ value at KL$>>$1, denoting the presence of additional high frequency components (at KHz range) for the myofilament suspensions in the activated state. Congo Red, which can shorten isolated myofibrils (Shih-fang Fan, Sci. Sinica 13:692, 1964), also increased the $\bar{\Gamma}$ value at KL$>>$1. After heat denaturation (42-44°C, 10 min.) or cleavage of the S_1 moiety with papain, Ca^{++} did not increase the $\bar{\Gamma}$ value. The dramatic increase in $\bar{\Gamma}$ at high values of KL could also be suppressed by treating the filaments with a myosin ATPase inhibitor such as vanadate ions (10 mM), or by replacing ATP with Cr-ADP. Our results revealed that (1) Ca^{++} could activate the active cross-bridge motions in isolated Limulus thick myofilaments, (2) the activated cross-bridges moved in a correlated way with frequencies in the KHz range and (3) actin is not essential for such active cross-bridge motions.

T-AM-Pos56 CONTRACTILE RESPONSES TO MgATP AND pH IN A THICK FILAMENT REGULATED MUSCLE: STUDIES WITH SKINNED SCALLOP FIBERS. R.E. Godt and J.L. Morgan. Dept. of Physiology; Medical College of Georgia; Augusta, GA 30912.

The striated adductor of the Atlantic deep sea scallop (Placopecten magellanicus), a thick filament regulated muscle, is reported to contain little or no troponin. We examined the effect on calcium activation of two agents (MgATP and pH) that alter the contractile threshold of thin filament regulated muscle, presumably through effects on troponin, to see if they also alter that of a thick filament regulated muscle. Small bundles of fibers from the striated adductor muscle were chemically skinned with Triton X-100 and saponin. We find that decreasing MgATP from 2 to 0.1 mM shifts the force-pCa curve of Placopecten skinned fibers to the left by about 0.8 log units (i.e. Ca^{2+} sensitivity increases some six-fold). Under similar conditions the force-pCa relation of frog skinned fibers shifts leftward by almost the same amount, 0.7 log units. The relative force-pCa curve of Placopecten fibers was unaffected by a decrease in pH from 7 to 6.5. This is especially interesting because decreasing pH is reported to decrease markedly the calcium affinity of Placopecten myofibrils (Chantler et al.; Biochem. 20:210, 1981). Moreover the force-pCa curve of similarly prepared skinned fibers from the Pacific pink scallop (Chlamys hastata hericia) shifts to the right by 0.5 log units (i.e. Ca^{2+} sensitivity decreases three-fold) with a pH decrease from 7 to 6.5. (These data on Pacific scallops are similar to unpublished observations on the same animal by S.K.B. Donaldson). Thus the molecular details of thick filament regulation appear to be more complex and varied than we had hitherto supposed. Supported by U.S.P.H.S. grant AM 31636. R.E.G. is an Established Investigator of the American Heart Assoc.

T-AM-Pos57 IS Ca^{2+}-TROPONIN AFFINITY FORCE-DEPENDENT? Franklin Fuchs and Robert Planchak. Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Recent studies with skeletal, cardiac, and barnacle muscle (Edman, 1981; Allen and Kurihara, 1982; Ridgway and Gordon, 1981) suggest that the affinity of Ca^{2+} for troponin-C may be a function of the force generated by attached cross-bridges. To test the hypothesis that Ca^{2+} binding is a force-dependent parameter we have measured the binding of Ca^{2+} to detergent-glycerol extracted rabbit psoas fibers under conditions in which cross-bridge cycling can be reversibly inhibited in the presence of Ca^{2+}. For this purpose we have used the ATPase inhibitor Na vanadate, since the vanadate (Vi) anion forms a reversible M·ADP·Vi complex which apparently remains dissociated from actin regardless of $_{p}Ca$ (Magid and Goodno, Biophys. J. 37:107a, 1982). At $_{p}Ca$ 5 psoas fiber contraction was strongly inhibited by Vi, with half maximal inhibition at 60 μM Vi. Using a double isotope procedure, Ca^{2+} binding curves were obtained over the $_{p}Ca$ 5-7 range with contracting fibers and with fibers maintained in a relaxed state by 1 mM Vi. Activating solutions otherwise contained 100 mM KCl, 5 mM $MgCl_2$, and 5 mM MgATP. The Ca^{2+} binding curves for the two groups of fibers were identical. These data suggest that force-generating cross-bridges, unlike rigor cross-bridges, do not influence Ca^{2+}-troponin affinity. (Supported by NIH grant AM10551).

T-AM-Pos58 EFFECT OF AMPPNP AND PP_i ON THE MECHANICAL PROPERTIES OF CONTRACTING MUSCLE. R. Cooke, Department of Biochemistry and Biophysics, Univ. of California, S.F. 94143 and E. Pate, Dept. of Mathematics, W.S.U., Pullman, Wa. 99163.

Isometric tension and stiffness have been measured for glycerinated, rabbit psoas muscle fibers as a function of ATP concentration in the presence and absence of the ligands AMPPNP and PP_i. The muscle is mounted between a force transducer (Akers, resonant freq.=2kHz) and a light, stiff beryllium arm connected to a rapid motor for changing muscle length (General Scanning). Stiffness measurements were made using a sinusoidal oscillation at a frequency of 500Hz and a .1% change in mucle length. At 2mM ATP, no change in stiffness or tension was observed in the presence or absence of 2mM PP_i. An increasing effect was observed with decreasing ATP concentration. At 25 micromolar ATP, 2mM PP_i resulted in a 30% decrease in the isometric tension while only a 10% decrease in the stiffness, when compared to that observed in the absence of ligand. Similar results were obtained using AMPPNP. The presence of nonhydrolyzable ligands which compete with ATP shifts the population of attached cross-bridge states. Since the decrease in stiffness and the decrease in tension in the presence of ligands is not correlated, we conclude that different cross-bridge states produce different tensions and different degrees of stiffness. The ligand induced decrease in velocity of contraction is consistently less than or equal to the decrease in tension. This implies that very efficient mechanisms exist for the dissociation of these ligands at the end of the power stroke in isotonically contracting muscle. This work was supported by grants to R.C. from USPHS, HL16683 and AM00479, and by grants to E.P. from NSF, PCM8208292, and from the Research and Arts Committee, W.S.U.

T-AM-Pos59 LENGTH-TENSION RELATIONSHIP OF SINGLE MYOFIBRILS

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Using a newly developed instrument for measuring the mechanics of single myofibrils (Iwazumi, Biophys. Soc. Abstr. 37:357a, 1982), the length-tension relation of single (or double) myofibrils from bull frog atrial cells has been measured at pCa values of 8.0, 6.0, 5.5, and 5.0. Main advantages of using single myofibril preparations over much larger multi-myofibril preparations are: 1. every sarcomere in the preparation is observable, thus enabling detection of a single abnormal sarcomere; 2. no effect of sarcoplasmic reticulum which exerts strong effects on sarcoplasmic Ca^{++} concentration even if Ca^{++} is buffered by EGTA; and 3. no activation delay due to Ca^{++} diffusion time. Bull frog atrial cells contained only two, sometimes only one, myofibrils in their tapered ends; therefore, suitable for extraction of the preparation after detergent treatment. The myofibril length was typically 100μm at rest under slightly taut condition. The basic protocol was to stretch the myofibril to predetermined lengths while at rest, then the relaxing solution was switched to one of contracting solutions. The solution flow was servo controlled for extreme stability. The active isometric tension increased with stretch up to the sarcomere length of 3.6μm when pCa was 6.0 and 5.5 but nearly constant when pCa was 5.0. There were no sarcomere oscillations which were often observed in much larger myofibril bundles activated by about pCa 6.0 solution. The myofibril failed suddenly and generated much less tension when some of the sarcomere exceeded the length of 3.6 μm. Supported by Texas Heart Association.

T-AM-Pos60 EFFECT OF ADRENALINE ON THE LENGTH-TENSION RELATION IN SINGLE FIBERS OF FROG SKELETAL MUSCLE. M. C. Garcia and G. H. Pollack, Dept. of Anesthesiology and Div. of Bioengineering, RN-10, Univ. of Washington, Seattle, WA, 98195.

At long sarcomere lengths tetanic tension develops very slowly. In some cases, instead of settling at a plateau, tension begins a slow decay just after having reached a maximum (Gordon, A. M., Huxley, A. F. and Julian, F. J., J. Physiol. 184:170-192, 1966; ter Keurs, H. E., Iwazumi, T. and Pollack, G. H., J. Gen. Physiol. 72:565-592, 1978). If such a decay in fact has an early onset, just after stimulation, it is possible that this decay masks the true maximum tension. If so, the shape of the length-tension relation may be affected. In order to reduce fatigue we employed adrenaline, a known potentiating agent (Gonzalez-Serratos, H., Hill, L. and Valle-Aguilera, R., J. Physiol. 315:267-282, 1981). Single muscle fibers from the tibialis anterior muscle of Rana temporaria were isolated and illuminated with a laser beam. Sarcomere length was estimated by the position of the first order of the diffraction pattern. Adrenaline (1×10^{-4} M), and in some cases isoproterenol, was added to the Ringer's solution immediately prior to the experiment. Adrenaline produced a modest potentiation of tetanic tension that decreased with sarcomere length. At 2.3 μm the mean potentiation was 9%; at 2.8 μm it was 6%; and at 3.1 μm it was negligibly small. Analysis of sarcomere length dynamics during tension development revealed little or no difference with or without adrenaline. The effects of isoproterenol on the length-tension relation were similar to adrenaline. Despite the length dependent potentiation induced by catecholamines, the effect was sufficiently small that the overall shape of the length-tension relation was not changed appreciably.

T-AM-Pos61 V_{MAX} IN STIMULATED VS. UNSTIMULATED MUSCLE FIBERS. F.V. Brozovich, J.W. Lacktis, and G.H. Pollack. Univ. of Washington, Dept. of Anesthesiology and Bioengineering, RN-10 Seattle, WA 98195.

We have found sarcomere shortening steps in both stimulated (Pollack et al., Nature 268: 757-759, 1977) and unstimulated (Lacktis et al., Biophys. J. 37: 235a, 1982) muscle fibers. To determine if the kinetics of shortening at zero load were different in stimulated and unstimulated fibers, we measured sarcomere shortening velocity during steps; i.e., the velocity during the periods of rapid shortening between pauses. Sarcomere length was computed on-line from the striation image using a phase-locked loop measuring system. Initial sarcomere length was 2.5 μm. In stimulated muscle, V_{max} of sarcomere shortening steps was determined by measuring the step velocities in contractions at loads of 0.75, 0.50, and 0.33 P_o and then extrapolating to zero load. In unstimulated fibers, V_{max} was determined by calculating shortening step velocities after a quick release of the resting fiber from its resting tension to zero load. V_{max} of sarcomere shortening steps in stimulated fibers was 4.8 l_o/sec while in unstimulated fibers it was 2.7 l_o/sec. These values are significantly different ($p>0.05$). These differences in V_{max} imply that if shortening in stimulated and unstimulated fibers occurs by the same process, the kinetics are apparently somewhat different.

T-AM-Pos62 CROSS-BRIDGES IN RELAXED AND ACTIVATED SKINNED MUSCLE FIBERS. M. E. Cantino and G. H. Pollack. Univ. of Wa., Depts. of Bioengineering and Anesthesiology, RN-10, Seattle, Wa, 98195.

Ultrarapid freezing has considerable potential as a method for preserving tissue ultrastructure without the use of chemical fixatives. In order to study the structure of unfixed myofilaments we have used a propane jet freezing device (based on a design by Müller, Meister and Moor, Mikroskopie 36: 129; with improvements by J. Gilkey and A. Staehelin) for cryofixation of relaxed and activated skinned muscle fibers. Single, mechanically skinned, R. temporaria semitendinosus fibers were mounted in relaxing solution between two hooks, over a specially prepared Balzers freeze fracture support (hollowed out and with a shallow groove milled in the top). A similar support was then clamped over the fiber and excess solution was removed. This sample was dropped between two jets of supercooled liquid propane. Other samples were first activated by placing a drop of relaxing solution containing 4-5 mM $CaCl_2$ on the fiber. After separating the two supports, the frozen tissue was fractured, deep-etched, and replicated in a Balzers freeze fracture unit. Replicas were examined in a JEOL 100C transmission electron microscope. Thick filaments displayed two distinct appearances. Most thick filaments in relaxed fibers had large areas with distinctly helical structure in the H-zone, with helical pitch of 42-43 nm and subunit axial repeat of 13-14 nm. Total filament diameters in helical regions were 25-33 nm. In activated fibers, this helical structure was much less prevalent. Instead, 16-20 nm filament cores were characterized by 20-30 nm projections (cross-bridges). Extended cross-bridges were also more common in the overlap region of relaxed fibers.

T-AM-Pos63 STEP SIZE AT DIFFERENT INITIAL SARCOMERE LENGTHS. J.W. Lacktis, F.V. Brozovich, and G.H. Pollack. Depts. of Anesthesiology and Bioengineering, RN-10. Univ. of Washington. Seattle, WA 98195.

Stepwise shortening of sarcomeres has been observed in actively contracting muscle using laser diffraction, cinemicrography, and on-line optical imaging techniques. Last year at this meeting we described experiments demonstrating stepwise sarcomere length changes in unstimulated muscle during stretch or release. In both stimulated and unstimulated fibers, step sizes ranged from 5 to 20 nm. We also found that certain step sizes were more frequently observed than others and that step sizes were seperated by 2 to 3 nm- that is, in a given experiment the most frequently occurring step sizes might be 9, 11, 13, 16 nm etc. The consistency of occurence of certain step sizes from both diffraction and imaging data suggested to us that step size might be related to some molecular repeat distance along the filaments. If this were the case, we might expect to see no dependence of step size on filament overlap up to sarcomere lengths of 3.65 μm. We observed stepwise length changes in unstimulated muscle fibers stretched or released from three initial sarcomere lengths, 2.2 μm, 3.0 μm, and 4.0 μm. Step sizes ranged between 5 and 20 nm in all three cases. These results were obtained using both laser diffraction and on-line imaging of striation patterns. We will discuss the relationship between step size and filament overlap and the implications of stepwise shortening beyond apparent overlap.

T-AM-Pos64 ROTATIONAL MOTION OF SPIN-LABELED MYOSIN HEADS DURING CONTRACTION: SATURATION TRANSFER EPR. Vincent A. Barnett and David D. Thomas, Dept. of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455

The development of an accurate model for the molecular mechanism of muscle contraction requires knowledge of the orientations and motions of myosin heads during contraction. In previous studies, conventional electron paramagnetic resonance spectroscopy (EPR) has been used to monitor the orientation of spin-labeled myosin heads in glycerinated rabbit psoas fibers during rigor, relaxation, and contraction (Thomas and Cooke (1980) Biophys. J. 32:891-906; Cooke, Crowder, and Thomas, Nature, in press). In the present study we have used saturation transfer EPR to detect the rotational motion of myosin heads, to determine whether the disorder observed previously in both relaxation and contraction is static or dynamic, on the microsecond time scale. Answer: dynamic. Probes on myosin heads are highly ordered ($\Delta\theta \leq 10^{o}$) and immobile ($\tau_c \geq 10^{-3}$ sec) in rigor but are highly disordered ($\Delta\theta \geq 90^{o}$) and mobile ($\tau_c = 10^{-6}$ sec -10^{-5} sec) in relaxation. This behavior in relaxation is also observed when myosin heads are detached from actin in the absence of ATP (Barnett and Thomas, submitted). During isometric contraction, both the conventional (orientation-dependent) and saturation transfer (motion-dependent) spectra appear to be linear combinations of the spectra in rigor and relaxation, implying that about 20% of the probes have the same rigid orientation as in rigor while the remainder are as disordered and mobile as in relaxation. The simplest explanation is that, under isometric tension, the probed region of myosin (SH-1) spends about 20% of its time attached to actin in the rigor orientation and the rest of its time detached and freely rotating. These probes show no evidence for more than one orientation of attached heads.

T-AM-Pos65 ORIENTATION OF SPIN-LABELED MYOSIN HEADS AT LOW IONIC STRENGTH IN RELAXED MUSCLE FIBERS. David D. Thomas and Toshio Yanagida*, Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455. *Present address: Faculty of Engineering Science, Osaka University, Osaka, Japan.

We have used electron paramagnetic resonance (EPR) to study the orientation of spin labels attached to myosin heads in relaxed fibers from rabbit psoas muscle. Conventional EPR spectra were recorded with fiber bundles oriented parallel to the magnetic field, resulting in a direct determination of the orientation distribution of probes with respect to the muscle fiber axis, and a less direct determination of the fraction of myosin heads attached to actin (Thomas and Cooke, Biophys. J. 32: 891-906, 1980). At high ionic strength ($\mu \geq 0.2$) and low temperature (2°C), the probes were highly disordered, indicating that all (> 95%) were detached from actin. When the ionic strength was decreased below 0.1, stiffness increased but tension was negligible, and a significant fraction of the probes (5-15%) was highly oriented, indicating attachment to actin. The orientation distribution of this attached fraction in relaxation was indistinguishable from that of rigor. Since the attached crossbridges should be at the beginning of the power stroke in relaxed muscle and at the end of the power stroke in rigor, the probed region of the myosin head does not appear to rotate during the power stroke. These results support previous results on contracting fibers (Cooke, Crowder, and Thomas, Nature, in press).

T-AM-Pos66 **"FREEZING" OF CARDIAC MYOFIBRILS IN THE "ON" AND "OFF" STATE BY CROSS-LINKING WITH GLUTARALDEHYDE.** R. John Solaro and Mark S. Rocklin*, University of Cincinnati, College of Medicine, Cincinnati, OH 45267.

We cross-linked cardiac myofibrils with glutaraldehyde in rigor conditions (80mM KCl, 20mM imidazole, pH 7, 0.1mM $CaCl_2$, 1mM $MgCl_2$) and in relaxing conditions (80mM KCl, 20mM imidazole, pH 7, 1mM EGTA, 3mM MgATP, 1mM free Mg). The myofibrils were incubated with 0.01% glutaraldehyde for up to one hour, and then washed by centrifugation and resuspension in 60mM KCl, 30mM imidazole, 2mM $MgCl_2$. In 7.5 min there was significant crosslinking of thin and thick filament proteins as judged by the ability of SDS treated myofibrillar preparations to penetrate polyacrylamide gels during electrophoresis. By 60 min the only bands visible on such gels were actin and tropomyosin. If the myofibrils had been crosslinked in rigor conditions, the MgATPase activity remained "on" regardless of the free Ca concentration. If the myofibrils had been crosslinked in relaxing conditions, the MgATPase activity remained "off" regardless of the free Ca concentration. The "on" activity and the "off" activity corresponded closely with the respective MgATPase activities at pCa 5 and pCa 8 of myofibrils processed in the same way as the crosslinked myofibrils but without glutaraldehyde. Myofilaments frozen in the "on" state bound Ca with greater affinity than myofilaments frozen in the "off" state. The Ca titration curve was shifted to the left in the "on" myofibrils by about one pCa unit, when compared to control or "off" myofibrils. Mikawa (Nature 278, 473, 1979) showed that the Ca regulated "on" conformation of reconstituted thin filaments can be frozen with glutaraldehyde. Our results indicate that this works in myofibrils. Our results also indicate that the structural changes associated with the Ca activation of the thin filament and with the reaction of crossbridge with the thin filament are associated with a change in the affinity of troponin C for Ca.

T-AM-Pos67 STUDIES OF HEME-LIKE MOLECULES BY SURFACE ENHANCED RAMAN SPECTROSCOPY by B. Simic-Glavaski, S. Zecevic and E. Yeager, Case Center for Electrochemical Sciences, Chemistry Department, Case Western Reserve University, Cleveland, Ohio 44106.

Metal free, cobalt and iron tetrasulfonated phthalocyanines (H_2-, Co-, Fe-TSPc) adsorbed on a silver electrode aqueous interface were studied in-situ by resonant and surface enhanced Raman spectroscopy and cyclic voltammetry. Electrode potential, elctrolyte pH, He and O_2 were used as experimental variables. Cycling voltammograms from 10^{-11} M/cm^2 adsorbed macrocyclic molecules indicate the four-step one-electron oxidation-reduction processes. The silver interface quenches fluorescence of H_2-TSPc and provides well resolved Raman spectra for a comparative analysis. A tentative Raman band assignment is provided. A similarity between Raman spectra obtained from solution phase and adsorbed phthalocyanine suggests physisorbed mechanism. Anomalously depolarized Raman spectra from the adsorbed phthalocyanines indicate edge-on model of the macrocyclic adsorption orientation. Some Raman bands show a frequency shift as a function of the electrode potential. These shifts correlate with the cyclic voltammogram oxidation peaks. The intensity is a function of the pH. Raman oxidation-reduction cycling (Raman ORC), behavior is discussed in terms of oxidation-reduction sites on the macrocyclic molecules. An interaction of O_2 with H_2-TSPc is followed by the breaking of the N double bond and in the case of Fe-TSPc shows the Bohr effect.

T-AM-Pos68 STRUCTURE AND PROPERTIES OF THE COMPLEX BETWEEN GLYCERALDEHYDE-3 PHOSPHATE DEHYDROGENASE AND BAND 3 IN THE RBC MEMBRANE. A.H. Beth*, B.H. Robinson+, C.E. Cobb*, S.D. Venkataramu*, J.H. Park* and W.E. Trommer^X. *Vanderbilt University, Nashville, TN 37232, +University of Washington, Seattle, WA 98195, ^{X}University of Kaiserslautern, FRG

Electron paramagnetic resonance studies on glyceraldehyde-3 phosphate dehydrogenase (GAPDH) spin labeled with analgoues of coenzyme NAD^+ have allowed correlation of structural and kinetic properties of both the soluble and red blood cell membrane bound enzymes. The soluble enzyme is a tetramer composed of four chemically identical subunits arranged as a dimer of dimers exhibiting C_2 symmetry. Each dimer has one tight and one loose binding site for coenzyme NAD^+. When coenzyme derivatives spin labeled at the 6 or 8 position of adenine were substituted for the natural coenzyme, the EPR spectrum showed resolved dipolar splitting of the resonances between each pair of spin labeled nucleotides bound within a given dimer of the GAPDH tetramer. Analysis of the dipolar spectrum indicated that the two labels were separated by about 12Å and that their principal nitroxide axes were nearly orthogonal. Comparison of the EPR spectra from soluble and membrane bound enzymes suggested that the tetrameric sturcture of the soluble dehydrogenase was preserved in the membrane complex with band 3 and that some coenzyme was displaced by the cytoplasmic N-terminal domain of band 3. The EPR lineshapes from enzyme bound and freely tumling labels indicated that between 1 and 2 coenzymes were released by the protein-protein interaction in intact ghosts. Enzyme kinetic data collected under similar conditions indicated partial inhibition of dehydrogenase activity which correlated with EPR results. Supported by NIH GM-07884 and The Chicago Community Trust/Searle Scholars Program.

T-AM-Pos69 MAGNETIC RESONANCE OF SPIN LABELED AEQUORIN, M. D. Kemple, G. K. Jarori+, B. D. Ray, B. D. Nageswara Rao, Physics Dept., IUPUI, Indpls., IN 46205 and F. G. Prendergast, Pharmacology Dept., Mayo Medical School, Rochester, MN 55901

Aequorin is a 20,000 molecular weight bioluminescent protein from the jellyfish Aequorea forskålea. Upon binding Ca^{2+} aequorin emits blue light (∿465nm) without a requirement for exogenous oxygen or other cofactors. Other ions such as Mn^{2+} can bind to aequorin and trigger the luminescence albeit at a lower rate. The single sulfhydryl group of aequorin is essential for its activity. We observed that covalently attaching the nitroxyl spin label, 4-maleimido-2,2,6,6-tetramethylpiperidino-oxyl, to aequorin at the sulfhydryl group inactivates the protein. The rotational correlation time of the label on the protein at 20°C in solution exceeds that of the free label in a dimethyl formamide solution by a factor of six as measured by EPR of the spin label. Mn^{2+} EPR shows that there is one tight binding site (K_D<<20μM) for Mn^{2+} on the spin labeled protein. The presence of bound Mn^{2+}, however, has no effect on the spin label EPR signal. The Mn^{2+} site is thus a substantial distance from the sulfhydryl group. The proton NMR spectrum of the spin labeled protein shows significant broadening of the aromatic resonances. (Supported by NSF grant PCM 8022075, NIH grant GM 30178 and a Petroleum Research Fund Grant, administered by ACS. Some NMR measurements were performed at the Purdue University biochemical magnetic resonance facility supported by NIH grant RR 01077.)

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T-AM-Pos70 SPIN FLUCTUATION RATES IN IRON PROTEINS MEASURED BY MÖSSBAUER SPECTROSCOPY. C. Schulz, Physics Dept., Knox College, Galesburg, IL 61401 and P. Debrunner, Physics Dept., University of Illinois, Urbana, IL 61801.

In paramagnetic iron proteins slow spin fluctuation is typical at 4.2K, and a static spin Hamiltonian $\mathcal{H} = D[S_z^2 - S(S+1)/3] + E(S_x^2 - S_y^2) + \beta H \cdot g \cdot S + S \cdot A \cdot I + \mathcal{H}_Q$ is adequate to parametrize the Mössbauer spectra. If measurements are extended to $T \gtrsim DS^2$ in order to have higher fine structure levels populated, intermediate spin fluctuation rates may complicate the analysis, and a dynamic lineshape model is needed. A simulation program incorporating spin fluctuation proved essential in the interpretation of the Mössbauer spectra of high spin ferrous proteins.[1,2] The formalism is based on Liouville operators and assumes that spin state transitions are dominated by one-phonon spin lattice processes.[2] We extended this formalism to the case of ferryl low spin (Fe^{4+}, $S = 1$) iron, such as compounds I and II of horseradish peroxidase, and to ferric high spin iron (Fe^{3+}, $S = 5/2$), such as native peroxidase and oxidized rubredoxin. For the $S = 5/2$ Kramers system we model the dynamic spin Hamiltonian by the spin anticommutator matrix.[3] Orbach processes alone yield good simulations at $T < 50K$, but Raman processes may be needed to explain the rubredoxin data for $T > 50K$. Supported in part by USPH GM16406.

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2 Winkler, Schulz and Debrunner, Physics Lett. 69A, 360-363 (1979).
3 Mattuck and Strandberg, Phys. Rev. 119, 1204 (1960).

T-AM-Pos71 HYDRATION AND CROSS-RELAXATION RATES BY NMR RELAXATION FOR MODERATE-SIZE PROTEINS IN SOLUTIONS WITH SALT. H. Pessen, T. F. Kumosinski and H. M. Farrell, Jr. Eastern Regional Research Center, USDA, Philadelphia, PA 19118.

Previous work from this laboratory has indicated that the concentration-dependent excess proton relaxation rates $R_1(\equiv T_1^{-1})$, for the spin-lattice, and $R_2(\equiv T_2^{-1})$, for the spin-spin mode, in β-lactoglobulin solutions with moderate salt concentrations are proportional to the total hydration $\bar{\nu}$, with either an isotropic or anisotropic model for the binding of water. In one approach to limiting the effect of cross-relaxation, R_1 and R_2 in both H_2O and D_2O were obtained for molecular aggregation states of the protein in solution and were interpreted on the basis of minimal intermolecular contributions of proton R_2. A second approach, based on proton NMR with a model of isotropic binding alone, takes into account intermolecular (R'_{2b}) in addition to intramolecular (R_{1b} and R_{2b}) contributions to the relaxation rates of bound water, and the cross-relaxation rate κ (all as functions of the correlation time τ_c), as well as the number $\underline{n}$ of surface protein protons cross-relaxing with bound water, and the protein concentration $\underline{c}$. With the assumptions only that (1) the τ_c distribution for bound water is narrow and (2) the intermolecular distance, for moderate-size protein molecules, is about 2.7 Å, and the protein relaxation rate $R_p >> n\kappa\bar{\nu}$, it is shown that $(dR_1/d\underline{c})_\mu = (R_{1b} + \underline{n}(\kappa + R'_{1b}))$ and $(dR_2/dc)_\mu = \bar{\nu}(R_{2b} + \underline{n}R'_{2b})$. Three such equations (for $dR_1/d\underline{c}$ at two frequencies and for $dR_2/d\underline{c}$ at one frequency) can be solved simultaneously, to give τ_c, $\bar{\nu}$, and $\underline{n}$. This treatment applied to β-lactoglobulin is compared with the results from the D_2O measurements for an assessment of the merits of the isotropic and anisotropic models.

T-AM-Pos72 IDENTIFICATION OF TERTIARY STRUCTURAL TYPES ALL-α, ALL-β, α + β AND α/β FROM CIRCULAR DICHROISM OF PROTEINS. P. Manavalan and W. C. Johnson, Jr., Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331.

Analysis of circular dichroism spectra of a number of proteins indicates that the spectra are not only sensitive to secondary structural elements, but also to tertiary folding patterns (all-α, all-β, α + β and α/β). For this purpose, each spectrum was analyzed in terms of (i) the positive band positions, (ii) negative band positions, (iii) crossover from negative to positive side and (iv) crossover from positive to negative side. The results indicate that each structural type can be differentiated simply on the basis of these four criteria. For instance, two distinct negative band minima around 209 nm and 220 nm and a crossover from positive to negative side around 172 nm is indicative of all-α type structure, whereas the spectrum with the same band positions, but the crossover above 175 nm is indicative of α/β structural type. The analysis also reveals that there are three different spectra representing all-β type protein structures. The major criteria for each such type and also for other structural classes are discussed. Based on these results, predictions are made for the additional proteins, which were not included in the analysis.

T-AM-Pos73 FLUORESCENCE STUDIES OF THE INTERACTION OF BASIC DRUGS WITH HUMAN OROSOMUCOID. H. B. Halsall and T. L. Kirley, Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221.

The human serum glycoprotein orosomucoid (OMD) binds a wide variety of ligands, principally at a binding ratio of 1 mole per mole. Current data suggest that the binding domains for these ligands have some common subsites. Quenching experiments in the absence and presence of drug were carried out with I^-, Cs^+, acrylamide and Cs^+, and acrylamide, respectively. At 37°C in the absence of drug, all of the fluorophores were accessible to acrylamide, whereas approximately 1/3 of the fluorescence was accessible to Cs^+ and essentially none was accessible to I^-. The Stern-Volmer plot for Cs^+ quenching curved downward indicating the possibility of selective quenching of a fluorophore. Saturation of OMD with chlorpromazine resulted in quenching of 60% of the intrinsic fluorescence. Quenching in the presence of chlorpromazine indicated overlap in the fluorescence quenched by the ligand and that quenched by Cs^+. These data expand our model of the binding site for chlorpromazine as containing a "buried" tryptophan, not accessible to Cs^+, and a second, solvent accessible tryptophan situated close to a negative charge. This negative charge may interact with the positively charged "arm" of the chlorpromazine ligand and other basic drugs. This additional electrostatic interaction could also explain the decreased temperature dependence of the binding of chlorpromazine and other basic drugs to OMD, in comparison with steroid binding, which falls off dramatically with increasing temperature, and is hydrophobic in nature.

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T-AM-Pos74 ^1H NUCLEAR MAGNETIC RESONANCE STUDIES OF PORCINE INTESTINAL CALCIUM BINDING PROTEIN. Judith G. Shelling, Brian D. Sykes, Joseph D.J. O'Neill† and Theo Hofmann†. MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta. †From the Department of Biochemistry, The University of Toronto, Toronto, Ontario.

^1H nuclear magnetic resonance (NMR) has been employed to study the environment of several proton nuclei (primarily those arising from aromatic residues) of the porcine intestinal calcium binding protein (ICaBP). An assignment for the single tyrosine (TYR 16) residue has been made on the basis of laser photochemical induced dynamic nuclear polarization (CIDNP) and homonuclear decoupling experiments. pH titration studies have shown that the tyrosine pK_a is unusually high in the apo protein, and increases even further upon the addition of calcium. However, the observation of a CIDNP effect with this single tyrosine in both the presence and absence of calcium indicates that this tyrosine is solvent accessible and therefore exposed on the surface of the molecule. Under the conditions of these experiments, the protein was observed to bind calcium with a 2:1 stoichiometry, at a rate of exchange slow enough that the NMR spectra are in the slow exchange limit. The presence of upfield shifted phenylalanine and methyl resonances in the apo protein indicate that there is a well-defined tertiary structure in the absence of calcium.

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T-AM-Pos75 STUDIES OF LIGAND BINDING TO ALCOHOL DEHYDROGENASE WITH DECAY-ASSOCIATED FLUORESCENCE SPECTROSCOPY. **Jay R. Knutson, Dana G. Walbridge, and Ludwig Brand, Biology Department, The Johns Hopkins University, Baltimore, MD 21218.**

Auramine O binds at the active site regions of horse liver alcohol dehydrogenase and quenches the intrinsic tryptophan fluorescence of the protein. Native HLADH exhibits biexponential behavior with decay times of 3.8 ns and 6.8 ns. Decay associated fluorescence spectroscopy, DAS (Biochemistry 21, 4671 (1982)) has indicated that the short decay component is associated with a fluorescence emission spectrum which is blue shifted as compared to that associated with the 6.8 ns decay. When auramine O is added, a third, shorter-lived (~0.5 ns) decay component is observed. The amplitide (alpha) of this new component increases with auramine O concentration. The Decay-Associated Spectrum of the 0.5 ns decay component is similar to that of the 3.8 ns component. The results are consistent with dynamic quenching of the 'buried' tryptophans by auramine O, probably via energy transfer. If quenching of these trp residues occurred independently for each enzyme subunit, the ratio alpha(3.8)/[alpha(3.8) + alpha(0.5)] should drop linearly with saturation. The actual behavior is quadratic and is consistent with dynamic quenching of both subunits by a single ligand.

Decay-associated fluorescence spectroscopy may thus be used to assess the influence of ligand binding on individual aromatic residues in a protein molecule. Either emission spectra (DAS) or excitation spectra (EDAS) may be obtained with the aid of signatory decay times. Other applications of decay associated spectroscopy to protein-ligand interactions will be discussed, along with the theory for analogous studies using harmonic and steady-state fluorescence methods. (Supported by USPHS GM 11632 and a fellowship to JRK from the Pharmaceutical Manufacturers' Association Foundation).

T-AM-Pos76 ON THE APPLICABILITY OF THE HILL TYPE ANALYSIS TO FLUORESCENCE DATA. Z.Grabarek and J.Gergely, Department of Muscle Research, Boston Biomedical Research Institute, Department of Biological Chemistry, Harvard Medical School and Department of Neurology, Massachusetts General Hospital, Boston, MA, 02114

In connection with our studies on the troponin-tropomyosin-actin interaction we have analyzed a fluorescence change f as a function of cation concentration C for a system containing a single fluorescent probe and two cation binding sites, using the following equation:

$$f = \Delta F / \Delta F_{max} = [2\beta KC + \alpha(KC)^2] / [1 + 2KC + \alpha(KC)^2] \quad (1)$$

where K is the binding constant of a single metal to the apoprotein, α is the increase in affinity for the second cation when the first site is occupied and β reflects the relative contribution of the single and double occupancy species to the total fluorescence change. If no cooperativity of binding occurs $\alpha = 1$; $\alpha < 1$ or $\alpha > 1$ for negative or positive cooperativity, respectively. Values for β are between 0 and 1; $\beta = 0$ if the species with one bound metal do not contribute to f and equals 1 if binding of the first cation is sufficient for producing the total fluorescence change. Fluorescence data calculated with various α and β values using (1) were fitted with the Hill equation $Y = KC^n/(1+KC^n)$. With no cooperativity in binding, a Hill coefficient (n) between 1-1.2 is obtained depending on β. In the range of α corresponding to the negative and low positive cooperativity, the transition midpoint and n depend very much on β. In case of positive cooperativity ($\alpha > 10$), however, the model of the fluorescence change applied has no effect on the relation between α and n. Values of $n > 1.4$ (for two binding sites per molecule) indicate an existence of positive cooperativity of binding regardless of the mechanism of the fluorescence changes. Supported by grants from NIH (HL-5949), NSF and MDAA.

T-AM-Pos77 TRYPTOPHAN FLUORESCENCE INTENSITY AND ANISOTROPY DECAY IN GLOBULAR PROTEINS. Duane P. Flamig and Bruce Hudson, Department of Chemistry and Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The doubled output of a synchronously pumped dye laser has been used in conjunction with single photon counting detection to obtain polarized time resolved fluorescence data for a variety of globular proteins (the number of tryptophans is given in parentheses): RNAse T1 (1), ACTH (1), casein (2), casein treated with carboxypeptidase A (1), HSA (1), BSA (2) and the S1 fragment of rabbit skeletal muscle with and without added ATP. The ACTH data have been obtained at 4, 10 and 20°C. In all cases the emission wavelength has been selected in a narrow band centered at 313, 337, 382 or 405 nm. Multiple exponential decay is observed in all cases. The decay components are a function of the emission wavelength. The interpretation of this behavior in terms of multiple protein states or excited state relaxation will be discussed. The polarization anisotropy decays are also a function of the emission wavelength, especially at short times. The typical pattern is one of an increased amplitude for a fast component when the anisotropy is measured at longer wavelengths.

Technical aspects of the analysis of subnanosecond fluorescence data will be discussed including the use of the emission of quenched tryptophan in solution as an appropriate instrument response function and the method of least squares analysis.

One of the goals of this work is an eventual comparison of the anisotropy decay with expectations based on structural determinations and simulations. There is, however, an ambiguity in the polarization of the emission dipole of tryptophan which makes this comparison difficult.

T-AM-Pos78 SECONDARY STRUCTURE MEASUREMENT BY RAMAN SPECTROSCOPY; INTERFERON STRUCTURE. Robert W. Williams, Department of Biochemistry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814.

The Raman amide III spectrum of several proteins of known structure have been analyzed for secondary structure information using methods developed earlier to analyze amide I data [1]. The information present in the amide III region appears to be complementary to information in the amide I band. Preliminary results from spectra of deuterium exchanged proteins indicate that quantitative secondary structure measurements can be made of exchanged and non-exchanged domains.

The Raman spectrum of interferon α-A has been analyzed using amide I and III methods giving 76% ± 5% helix and 7% ± 3% beta strand. These results suggest that this protein contains more than four helical segments.

[1]R. W. Williams and A. K. Dunker (1981) J. Mol. Biol. 152, 783-813.

T-AM-Pos79 STUDY OF A RIBONUCLEASE FOLDING INTERMEDIATE AND OF HELIX TERMINATION IN A PEPTIDE FRAGMENT. David Brems, Peter S. Kim and Robert L. Baldwin, Biochemistry Dept., Stanford University Medical Center, Stanford, CA 94305.

The stability to amide proton exchange of the native-like folding intermediate I_N has been compared to that of native RNaseA (N) in pulse label experiments. Using conditions worked out by Schmid (private communication, 1982), we stop refolding when I_N is the major constituent. We can measure the stability of a given structure to exchange by determining the alkaline pH at which exchange first occurs in a 10-second pulse of D_2O or $(^3H)H_2O$. This has been done for a group of about 16 amide protons (as measured by ^{1}H-NMR) which show exchange above pH 8. Exchange occurs in a narrow pH range centered near pH 9 for N and near pH 8 for I_N, showing that I_N is less stable to exchange than is N. The technique should be applicable also to the study of folding intermediates which precede I_N. S-peptide (residues 1-20) of RNaseA shows partial α-helix formation in aqueous solutions near 0°C (Kim et al., 1982, J. Mol. Biol., in press). Here we show that the α-helix formed by isolated S-peptide is localized to certain residues. The ^{1}H-NMR chemical shifts of various sidechain groups have been measured as a function of temperature, [urea] and [GuHCl], based on the knowledge that unfolding of the helix formed by peptide 1-13 (lactone) can be monitored by sidechain chemical shifts (Bierzynski et al., 1982, PNAS 79, 2470). We show here that, whereas the chemical shifts of Thr 3 (γCH_3), Phe 8 (ϕ-H) and His 12 (C2H) change with temperature and denaturants, the chemical shift of Thr 17 (γCH_3) does not change significantly with temperature or denaturants, indicating that the α-helix does not extend as far as residue 17. The nature of the stop signal is under study.

T-AM-Pos80 VIBRATIONAL ANALYSIS OF L,D PEPTIDES: STRUCTURAL ANALOGUES OF GRAMICIDIN A. V. Naik and S. Krimm, Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109.

We have obtained ir and Raman spectra of Boc-(L-Val-D-Val)$_n$-OMe, with n=4,6, and 8. A single crystal x-ray analysis of the n=4 compound [Benedetti, et al, Nature 282, 630 (1979)] shows that it forms a double-stranded β-helix with antiparallel chains (type $\beta^5_{DL}{}^6$), which would be analogous to one of the structures proposed for gramicidin A. Powder x-ray diffraction patterns of the n=6 compound suggest that it has a structure analogous to that of the n=4 molecule. The spectra of these three compounds are very similar in the solid state, indicating that their structures are similar. There are small shifts in the Raman amide I mode, from 1678 cm^{-1} for n=4 to 1672 cm^{-1} for n=8, and in the ir amide I mode, from 1644 cm^{-1} for n=4 to 1640 cm^{-1} for n=8. The ir amide II mode undergoes a larger shift, from 1540 cm^{-1} for n=4 to 1552 cm^{-1} for n=8, as does the amide V mode, from 670 cm^{-1} for n=4 to 700 cm^{-1} for n=8. These shifts suggest a strengthening of the peptide hydrogen bonds as the chain length increases, a conclusion that is supported by the decrease in the amide A frequency, from 3300 cm^{-1} for n=4 to 3280 cm^{-1} for n=8. This may result from a decreased influence of the end group as the chain gets longer, resulting in a more regular backbone structure. Normal mode calculations have been done for a canonical $\beta^5_{DL}{}^6$ double helix, and comparisons with the observed spectra will be discussed. This research was supported by NSF grants PCM79-21652 and DMR78-00753.

T-AM-Pos81 ELECTROPHORETIC LIGHT SCATTERING (ELS) STUDIES OF POLY(L-LYSINE)(PLL). J.P. Wilcoxon and J.M. Schurr, Department of Chemistry, University of Washington, Seattle, WA 98195.

ELS is employed to determine the electrophoretic mobilities μ_E and apparent diffusion coefficients D_{ELS} obtained from ELS linewidths for three samples of PLL of degree of polymerization n=406, n=946, and n=2273 as a function of salt (C_s) and polyion (C_p) concentration. Dynamic light scattering (DLS) is used to determine apparent diffusion coefficients D_{app} of these same PLL samples in the absence of the applied field. This polyelectrolyte system exhibits an ordinary to extraordinary phase transition at low salt concentrations that is manifested by a more than 50-fold decrease in D_{app} as well as a two-fold decrease in scattered intensity.

In the extraordinary phase the D_{ELS} values are typically very small, though still somewhat (frequently two-fold) larger than D_{app}. When the salt concentration is raised to enter the ordinary phase D_{ELS} remains small, less than one tenth of D_{app}, in the low-salt end of the ordinary phase, but increases with increasing C_s to become comparable to, though still about two-fold smaller than, D_{app} in 0.1 M NaBr. The fluctuations in polyion density responsible for the observed ELS signal are evidently relaxed very slowly under low-salt conditions, regardless of which phase prevails.

In the ordinary phase μ_E increases rapidly with decreasing C_s, but in the extraordinary phase μ_E either remains nearly constant for (n=946), or actually decreases (for n=2273), as C_s decreases further to 0.

The present μ_E data disagree with a previous empirical prediction of Schurr and rule out the particular interpretation of the diffusion viral coefficient upon which that was based.

T-AM-Pos82 INFLUENCE OF FIBRIN STABILIZATION ON CLOT STRENGTH. L. Shen[1] and L. Lorand[2], [1]Abbott Laboratories, Anti-infective Research Division, North Chicago, IL 60064; [2]Department of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, IL 60201.

The contribution of γ-glutamyl-ε-lysine isopeptide linkages to clot strength, brought about by the functioning of the fibrin stabilizing (Factor XIII) system in plasma (see Lorand, Ann. N.Y. Acad. Sci. 202, 1, 1972), is one of the important questions in blood coagulation. The static elastic moduli of human platelet poor plasma clots were examined by two independent approaches, employing a Couette type elastometer. (A) In the first set of experiments, primary amines were used which do not interfere with the aggregation of fibrin molecules, but are known to inhibit the formation of isopeptide bridges selectively (Lorand et al., Biochemistry 7, 1214, 1968). Addition of amines of different chemical structures and inhibitory potencies (hydroxylamine; aminoacetonitrile; 2,4-dinitrophenylcadaverine; mesitylenesulfonylcadaverine; dansylcadaverine; dansylthiacadaverine) to normal plasma caused a five-fold lowering of the elastic modulus. Accordingly, 20% of normal can be considered to represent the elasticity of a fibrin network formed in the plasma milieu without isopeptide side chain bridges. (B) In another set of experiments with a plasma specimen which genetically lacked Factor XIII, measured doses of the purified human Factor XIII zymogen were added. Maximally, a five-fold increase in the static modulus of the clot structure could be achieved. In view of the effects of amine inhibitors described above, this represents complete normalization of the hereditary deficiency. Supported by U.S.P.H.S. Research Career Career Award HL-03512 and by NIH grant HL-16346.

T-AM-Pos83 EFFECTS OF CROSSLINKING ON THE RIGIDITY AND PROTEOLYTIC SUSCEPTIBILITY OF HUMAN FIBRIN CLOTS. Ralph Nossal and Jules A. Gladner, National Institutes of Health, Bethesda, MD 20205.

Clots formed in reconstituted human plasma or from purified human fibrin were studied in order to assess the effects of subunit crosslinking on clot strength and on resistance to plasmin degradation. The relative amounts of α chain and γ chain ligation were varied by adding factor XIII to the samples. We observe that appreciable $\gamma - \gamma$ crosslinking always proceeds detectable formation of α dimer or α polymer. Non-invasive light scattering measurements of the shear modulus G(t) indicate that ligation of γ chains and of α chains have qualitatively similar effects on clot strength. Since α crosslinking occurs very slowly in the clots which are formed from plasma, we infer that under physiological conditions the involvement of α chains in the development of clot strength probably is only a secondary function. Experiments regarding the maintenance of clot rigidity in the presence of plasmin similarly do not show any special role for the α chains. Corroborative information has been obtained by using quasielastic light scattering techniques to study the size of particles shed from the surfaces of fibrin clots undergoing fibrinolysis.

T-AM-Pos84 BIOPHYSICAL STUDIES OF Cu-Zn SUPEROXIDE DISMUTASE - Alison Butler[+], Risa J. Peoples[+], Alex Avdeef[++], & Joan S. Valentine[+] - [+]Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90024, and [++]Department of Chemistry, Syracuse University, Syracuse, New York 13210.

Cu-Zn superoxide dismutase (SOD) is a dimeric protein comprised of two identical subunits of MW ~ 16,000. The metal binding region of each subunit contains Cu^{2+} and Zn^{2+} bridged by a histidyl imidazolate anion. X-ray crystal structural results indicate that the geometry around the Cu^{2+} is distorted square planar plus an axial water and around the Zn^{2+} is approximately tetrahedral. We have monitored the binding of Cu^{2+} to apo-SOD by Cu^{2+}-selective electrode, VIS-UV and ESR techniques. At pH 3.5 only two equivalents of Cu^{2+} bind to the native Cu site per protein dimer and no Cu^{2+} binds to the native Zn^{2+} sites. At pH 5.5 four equivalents of Cu^{2+} bind to the Cu & Zn sites (in acetate buffer) and the Cu^{2+} ions are bridged by imidazolate. Between pH 4-5, however, intermediate amounts of copper bind to the protein depending on the pH. VIS-UV and ESR evidence suggests that although copper is bound to the native Zn^{2+} site, its environment is similar to the native Cu^{2+} site and, moreover, that in this pH range the imidazolate bridge is broken.

We are also studying the mechanism of Cu^{2+} migration from a native Cu^{2+} site to a native Zn^{2+} site in the zinc-free protein when the pH is raised. The kinetics show biphasic behavior and the rates are approximately proportional to base concentration. The results will be discussed in terms of the metal migration as well as overall protein conformational changes.

T-AM-Pos85 ROLES OF SUBUNIT INTERACTIONS IN THE ALLOSTERIC REGULATION OF RABBIT MUSCLE PHOSPHOFRUCTOKINASE. Michael A. Luther, H.F. Gilbert* and James C. Lee. Dept. of Biochemistry, St. Louis University School of Medicine, St. Louis, MO, 63104 and McLean Dept. of Biochemistry, Baylor College of Medicine, Houston, TX, 77030.

The simplest mode of association of rabbit muscle phosphofructokinase (PFK) has been shown to be $M_1 \rightleftharpoons M_4 \rightleftharpoons M_{16}$ at pH 7.0, 23° C and low protein concentrations. In the presence of activators, inhibitors or buffer alone the data can be best fitted with M_4 assuming a sedimentation coefficient (S°_4) of 13.5 S. However, in the presence of substrates S°_4 has been demonstrated clearly by sedimentation velocity and active enzyme centrifugation techniques to be 12.4 S. Thus, it was proposed that the 12.4 S tetrameric PFK represents the active form whereas the 13.5 S form is inactive. The existence of the inactive tetrameric form has not been demonstrated experimentally.

Recently, Gilbert has shown that PFK can be reversibly inhibited by oxidized glutathione. The physical state of the enzyme is not known. Hence, sedimentation studies were conducted on this glutathione inactivated form. In the presence of saturating amounts of fructose-6-phosphate and the non-hydrolyzable ATP analog of AMP-PNP inactivated PFK sediments as a tetramer of 13.5 S. This effect can be reversed by the addition of DTT, which re-activates PFK. Upon addition of both substrates PFK sediments as a tetramer of 12.4 S, thus, demonstrating for the first time the presence of an inactive 13.5 S tetrameric PFK. It was further shown that inactivated PFK can still undergo self association with the same stoichiometry although K_4^{app} has decreased by four fold over that of the native enzyme under identical conditions. Conversely, citrate affects the sedimentation behavior of the inactivated form quantitatively in the same manner as that of the native enzyme.

T-AM-Pos86 DIFFERENCES IN RESPONSE TO L-LACTATE AND OTHER ALLOSTERIC LIGANDS BY HEMOCYANIN SUBUNITS OF THE SPINY LOBSTER. Bruce A. Johnson, Joseph Bonaventura, and Celia Bonaventura. Duke University Marine Laboratory, Beaufort NC 28516.

Many hemocyanins are composed of a mixture of electrophoretically distinguishable subunits. There is now considerable evidence for the specific role of individual subunit types in the assembly process of hemocyanins above the level of the hexamer. The hemocyanin of the lobster, Panulirus interruptus, is a heteropolymer but assembles only to the level of a hexamer. We have investigated the interaction of a variety of allosteric ligands with this hemocyanin and its purified subunits. Homogeneous hexamers, assembled from purified subunit types show specific responses to pH, Ca, Mg and lactate. Some, like the native molecule, show increased oxygen affinity in the presence of L-lactate (at constant pH), whereas others lack this effect. The Bohr effect is smallest with hexamers having highest levels of cooperativity . Unlike the situation reported for other crustacean hemocyanins, divalent cations decrease the oxygen affinity and magnesium is, in general, much more effective at this than calcium. The large differences shown in this system for related protein subunits should aid in understanding the molecular basis for allostery in the hemocyanins.

T-AM-Pos87 PROTEIN DIMENSIONALITY, CRYSTALLOGRAPHIC STRUCTURE, AND ELECTRON SPIN RELAXATION RATES. J. T. COLVIN, G. C. WAGNER, J. P. ALLEN, AND H. J. STAPLETON, Depts. of Physics and Biochemistry, University of Illinois, Urbana, IL 61801.

The fractal dimensions of polypeptide structures are estimated from the x-ray crystallographic coordinates of the α-carbon atoms. Dimensionalities of the protomer structures for 50 proteins are within the limits $1 \leq d \leq 2$ and range from 1.20 (immunoglobin Fab, L chain) to 1.81 (hexose kinase). Proteins of homologous supra-secondary and tertiary structures exhibit similar d-values. This result indicates the fractal dimension of a biopolymer, which originates as a fundamental parameter from polymer theory, may also provide a general parameter on which to base comparisons of protein structure. We have previously shown (1), that the temperature dependence of the Raman relaxation rate in spin 1/2 iron proteins appears to reflect their fractal dimension. In 6 different systems the observed temperature dependence is T**(3+2d) rather than T**9, which occurs in 3-dimensional solids. Relaxation data for cytochrome C551, metMb.OH, and cytochrome C yield d-values in good agreement with those calculated from x-ray data (1.43±.09/1.44±.04, 1.61±.05/1.53±.03, 1.66±.05/1.65±.05, respectively). Relaxation studies on putidaredoxin and C551 as a function of ionic strength indicate lower d values result in protein solutions of low ionic strength. These results seem to validate a recent correction to our model (2), which reduces d to 1 in the absence of off-backbone connectivity, and suggest a correlation between connectivity and ionic strength. Supported in part by NIH Grants GM24488, AM00562, and the University of Illinois.

(1) J. P. Allen, et al., Biophysical Journal 38, 299 (1982).
(2) S. Alexander and R. Orbach, J. Physique-Letters, 43, L-625 (1982).

T-AM-Pos88 SMALL ANGLE NEUTRON SCATTERING FROM NATIVE AND IRRADIATED SUPEROXIDE DISMUTASE IN AQUEOUS SOLUTION

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The approximate size and shape of holo and apo forms of bovine superoxide dismutase (SOD) were determined by small angle neutron scattering from aqueous solutions at neutral pH. A model assuming a cylindrical shape gave the best fit to the data for both forms of SOD. The radius of gyration, R_g, of the apoenzyme was found to be marginally larger than that of the holoenzyme. Scattering from the protein vanished for H_2O/D_2O mixtures containing 42(±2)% D_2O, and negligible dependence of R_g on D_2O fraction indicated uniform scattering density. Irradiation with ^{60}Co gamma rays resulted in aggregation of SOD molecules; scattering at small doses was interpreted in terms of pairwise side-by-side aggregation. For large doses ($\approx 3.8 \times 10^3$ Gy) and at relatively high enzyme concentration (320 μM), a time dependent decrease in some of the damage was found and this was interpreted to be due to the breakup of molecular pairs stacked end-to-end. The equilibrium concentration of separated subunits was found to be unobservable and calculations were carried out to show that effects of denaturation were negligible for the radiation doses employed (maximum 3.8×10^3 Gy). The results are consistent with HPLC data which show that the apo and holo forms of SOD interact with each other and that aggregates of irradiated SOD are readily dissociated, resulting in a single elution peak.

T-AM-Pos89 LOW RESOLUTION STRUCTURE OF FIBRINOGEN AND FIBRIN. J.W. Weisel*, C.V. Stauffacher, J. Fillers, G.N. Phillips, Jr.** and C. Cohen, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, *Department of Anatomy, University of Pennsylvania, Philadelphia, PA and **Department of Physiology and Biophysics, University of Illinois, Urbana, IL.

Analysis of crystals and microcrystals of protease-modified fibrinogen by electron microscopy and X-ray diffraction is beginning to reveal the complex shape of this large protein. The molecule has a projected length of 450Å and bonds end-to-end to form filaments that build these different structures. The overall molecular shape and end-to-end contacts appear to be conserved in virtually all arrays, including fibrin, but the lateral stagger between filaments differs. A low resolution structure for the molecule has been derived by computer simulation of electron micrographs of crystals, microcrystals and fibrin. The molecule is seen in more detail than the Hall-Slayter model and appears to be made up of seven globular domains connected by rod-like segments. This model is being refined against the three dimensional crystallographic data to provide starting phases for the low resolution structure determination. Using this model, detailed features of the band pattern of fibrin seen in the electron microscope can now be related to the molecular packing. This new picture of the organization of fibrin also clarifies the functional role of specific regions of the fibrinogen molecule in clot formation.

T-AM-Pos90 COLLAGEN STRUCTURE IN LAMPREY NOTOCHORD. Scott Sheren, Eric Eikenberry, and Barbara Brodsky. UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854.

The notochord is an axial supporting structure that is replaced by the backbone during development in higher animals but is retained throughout life in primitive vertebrates such as lamprey. In the notochord sheath collagen fibrils of small uniform diameter are oriented circumferentially around a central cylinder of vacuolated cells. We have studied the composition and structure of this collagen. The major collagen in the sheath is homologous to that in mammalian cartilage, Type II collagen, in terms of its high hydroxylysine content, its cyanogen bromide peptide pattern, and its charge distribution as visualized by electron microscopy of SLS crystallites. Small amounts of other collagen chains are also present which resemble the minor collagens found in cartilage. The melting temperature of both the major and the minor collagens is near 31°, a value that is correlated with a low imino acid content and the lower physiological temperature of this organism. X-ray diffraction of the sheath gives a pattern with the characteristic 67 nm meridional reflections found in collagenous tissues. The intensities of these reflections differ from those observed for tendon, and we are using Patterson and difference Fourier analyses to interpret these differences in terms of axial fibril structure. An equatorial diffraction pattern was also observed. The low angle equatorial maxima appear to be cylinder transforms of the collagen fibrils, which allows calculation of their diameters in the hydrated state. The higher angle equatorial maxima appear to be derived from ordered lateral packing of molecules in the fibrils, and may be indexable in terms of a lattice. These studies on notochord collagen will be of value in understanding the evolution and structural role of cartilage-specific collagens.

T-AM-Pos91 IN VIVO ASSEMBLY AND IN VITRO RENATURATION OF SKELETAL MUSCLE TROPOMYOSIN. H.R. Brown, D.D. Bronson and F.H. Schachat. Department of Anatomy, Duke University Medical Center, Durham, NC 27710 (Intro. by J.J. Blum)

Analysis of the dimeric forms of tropomyosin in rabbit skeletal muscle indicates that αβ is the preferred form. The prevalence of heterodimer in a simple coiled-coil protein was unexpected since, if assembly occurred on the polyribosomes, homodimers would be expected, while if association occured by unbiased association of free subunits, a random distribution of all three forms should have been found. To determine the molecular basis of the in vivo assembly process, we have attempted to find in vitro renaturation conditions which exhibit a substantial bias toward heterodimer formation. Of three processes analyzed, denaturation in urea followed by dialysis, heat denaturation followed by rapid cooling, and heat denaturation followed by slow cooling, none have shown a preference for αβ formation. At best, one yields a distribution consistent with random association, otherwise homodimers are favored. These results indicate that tropomyosin renaturation in vitro is different from the pathway of in vivo assembly. Coupling the results of this analysis with the published results on the unfolding of tropomyosin by Woods, by Williams and Swenson, and by Potekhin and Privalov, it appears that renaturation reactions which initiate at the most stable regions of the molecule result in the generation of homodimeric species.

This work was supported by a grant from the NIH (NS 18228) to F.H.S.

T-AM-Pos92 DETERMINATION OF POLYELECTROLYTE CHARGE BY DIALYSIS EQUILIBRIUM DURING VELOCITY SEDIMENTATION. Emory H. Braswell, The University of Connecticut, Storrs, Conn. 06268.

The molecular weight (MW) of a polyelectrolyte as experimentally determined by sedimentation, will be lower than the true value, even in the presence of salt. The extent of error depends on (among other factors) the effective (as opposed to titratable) charge (Z) on the polyelectrolyte, which can be determined by performing a dialysis equilibrium experiment in which the polymer is dialyzed against a salt-containing solvent. At equilibrium there will be a greater concentration of salt outside the dialysis bag, the magnitude of which is controlled by the concentration and Z of the polymer. Casassa and Eisenberg showed that one could correct the apparant MW obtained from sedimentation by incorporating the results of the dialysis experiment without explicitly calculating Z. The technique presented here is based on the fact that dialysis occurs across the boundary of a velocity sedimentation experiment. As expected, a higher salt concentration appears on the meniscus side of the boundary than that on the plateau side. It can be detected as a decrease in the refractive index difference between the solution and solvent (measured before the experiment) and that across the boundary. The latter is measured by Rayleigh interference, and the plates read (to $\pm$ 0.01 fringe) by an automated plate reader developed by D. Yphantis et al. The duration of the experiment (< 1 hr), the small quantity of material needed, coupled with the advantage that the experiment can be converted to one in which the MW of the polymer is obtained, makes this technique attractive. Data is presented from studies with Na, Ca and Cs salts of heparin. Internal agreement of the corrected MW and Z values attest to the validity and accuracy of the approach. This technique will be used to test various aspects of the Manning polyelectrolyte theory.

T-AM-Pos93 PROBING THE MACROMOLECULAR RECOGNITION SPECIFICITY OF THE SERINE PROTEASES,*M. N. Liebman T. F. Kumosinski+ and E. M. Brown+, Dept. of Pharmacology, Mt. Sinai School of Medicine, CUNY, New York, N. Y., 10029* and Eastern Regional Research Center, US Dept. Agric., Philadelphia, Pa. 19118+

The serine proteases are involved in a variety of biological control functions. This type of interaction, termed limited proteolysis, is responsible for enzyme cascades including complement activation, hormone activation and fertilization, and reflects the high degree of macromolecular specificity programmed into these enzymes by evolutionary constraints. The structural features responsible for the macromolecular specificity have been defined (Liebman); they include surface features of the enzyme molecules external to the active site itself. As a result, a new approach to enzyme inhibition is suggested, directed towards the individual enzyme's macromolecular recognition region, not the structurally homologous active site specificities. Analysis of the functional form of the recognition surface, e.g., van der Waal's and electrostatic potential surfaces, points to regions for specific site complementation and inhibitor design. Prior to genetic engineering attempts to design polypeptide inhibitors, we have probed these hypotheses by biophysical techniques (sedimentation equilibrium determination of molecular weights, differential circular dichroism of both the macromolecular components and resultant complexes, free energy analysis of complex formation, regulation of autolysis, evaluation of calcium ion binding, etc.) in order to examine macromolecular complexes and autolytic capabilities and to evaluate the potential design of inhibitors of these processes. The results are interpreted in terms of conformational changes, both within the active site and in other regions of the three-dimensional structure, including the macromolecular recognition surface. They support the recognition surface hypothesis and provide some of the bases for development of a genetic engineering program of inhibitor design. *Revson Found. Fellowship

T-AM-Pos94 SOLUBLE OLIGOMERS OF THE NEUROFILAMENT CORE PROTEIN. M.R. Lifsics and R.C. Williams, Jr., Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235

The 68,000 (68K) dalton polypeptide of mammalian neurofilaments assembles *in vitro* into 10 nm filaments and presumably forms the core of neurofilaments. We have found that purified 68K protein forms soluble oligomers in 10 mM Tris buffer and have characterized these species with respect to sedimentation coefficient, molecular weight, and secondary structure. Protein was purified from bovine spinal cord neurofilaments by DEAE and hydroxylapatite chromatography in 8 M urea. In 8 M urea the 68K polypeptide sedimented with an $S_{20,w}$ of 2.0, had a weight average molecular weight ($M_{w,app}$), determined by equilibrium sedimentation, of 69,000±2,000 daltons and had no apparent secondary structure detectable by CD spectroscopy. Urea was removed by exhaustive dialysis against 10 mM Tris, pH 8.5, 1 mM EGTA and 0.1 mM DTE. Protein was soluble to 1.6 mg/ml in this buffer. Upon removal of the denaturant the polypeptide acquired a molar ellipticity value of -30,000 deg.-cm^2/decimole at 208 nm, indicative of extensive regions of α-helix. At low protein concentrations (0.2 mg/ml) the protein sedimented as a single boundary with an $S_{20,w}$ of 4.8. It became increasingly heterodisperse at higher protein concentrations. $M_{w,app}$ was determined by equilibrium sedimentation on two different preparations at six initial concentrations. Results indicate that the solutions were heterogeneous and non-ideal. Values of $M_{w,app}$ ranged upward from 210,000 daltons, suggesting (Yphantis, Biochemistry 3:297, 1964) that the species of lowest molecular weight is a trimer of the 68K polypeptide that may correspond to a three chain coiled-coil described in intact 10 nm filaments (Steinert et al., PNAS 77:4534, 1980). Supported by NIH grants GM 29834 and 2T 32 GM07319.

T-AM-Pos95 INTERACTION OF THE NUCLEOSOMAL CORE HISTONES: A CALORIMETRIC STUDY OF OCTAMER ASSEMBLY Robert C. Benedict, Evangelos N. Moudrianakis, and Gary K. Ackers, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218 (intro. by Warner E. Love)

Heats of assembly for histone subunits into the nucleosomal core octamer have been studied by isothermal heatburst microcalorimetry. The assembly follows a "mixed association" reaction scheme [Eickbush, T. H. and Moudrianakis, E. N. (1978) Biochemistry 17, 4955]:

$$D + T \longleftrightarrow DT \quad \text{(hexamer)} \qquad (1)$$
$$DT + T \longleftrightarrow D_2T \quad \text{(octamer)} \qquad (2)$$

where the H2A-H2B dimer is denoted by D and the $(H3\text{-}H4)_2$ tetramer by T. The standard enthalpies for reactions (1) and (2) are found to be large and unequal at pH 7.5, 2M NaCl, 25°C ($\Delta H^0_1 = -30.8$ kcal; $\Delta H^0_2 = -22.5$ kcal). Previous results indicate a cooperativity in the assembly process: the intrinsic equilibrium constant for reaction (2) being about four times as great as that of reaction (1) [Godfrey, J. E., Eickbush, T. H., and Moudrianakis, E. N. (1980) Biochemistry 19, 1339]. We find that the calorimetric heats in different buffers show no net protonation change for overall octamer assembly, but indicate a compensating sequential release and absorptions of protons for the two-step reaction. An independent titration experiment confirms the compensating protonation effects. The nature of the dominant interactions stabilizing the octamer are discussed along with the relationship of these findings to possible cooperative interactions within the nucleosome. Supported by grants from the NSF and NIH.

T-AM-Pos96 BLOOD COAGULATION FACTOR Va: MOLECULAR WEIGHT, SHAPE AND SUBUNIT INTERACTIONS. Thomas M. Laue[1], Arthur E. Johnson[1], Charles T. Esmon[2] and David A. Yphantis[3]. [1]Department of Chemistry, University of Oklahoma, Norman, OK, 73019; [2]Section of Hematology and Thrombosis, Oklahoma Medical Research Foundation, Oklahoma City, OK, 73104; [3]Biochemistry and Biophysics Section, Biological Sciences Group, University of Connecticut, Storrs, CT, 06268.

We have used the analytical ultracentrifuge to investigate blood coagulation Factor Va and its two non-identical subunits, Vl and Vh. The following were determined in 50mM Tris (pH 7.6), 0.1M NaCl, 1mM benzamidine and either 2mM EDTA or 10mM Ca^{++}: 1) Vl subunit M=82,000, $s^{o}_{20,w}$=5.12 S, prolate axial ratio 2.8:1 (assuming 0.3g H_2O/g protein), 2) Vh subunit M=92,000, $s^{o}_{20,w}$=5.39 S, prolate axial ratio 4.2:1 and 3) the Va molecule (in 10mM Ca^{++}) M=170,000, $s^{o}_{20,w}$=8.34 S, prolate axial ratio 3.5:1. The data are consistent with a Va molecule that is composed of one Vl and one Vh subunit arranged side-by-side rather than end-to-end. The Vl and Vh subunits each undergo a self-association that is weak, reversible and independent of the Ca^{++} concentration. The Vl self-association has a monomer-dimer association constant near 10^4 M^{-1}. Higher order complexes are observed but their stoichiometry cannot be assigned unambiguously. The Vh subunit exhibits a monomer-dimer association constant near 10^3 M^{-1}. For an equi-molar mixture of Vl and Vh in the presence of 2mM EDTA, we observed no association that could be ascribed to the formation of a Vl-Vh complex. However, in the presence of 10mM Ca^{++} hetero-association of the Vl and the Vh subunits was strongly evident, indicating that the single Ca^{++} bound per Va molecule exerts a profound affect on the association behavior of the two subunits.

T-AM-Pos97 MOLECULAR WEIGHT OF THE CP-II PROTEIN OF SPINACH THYLAKOID MEMBRANES. Guy Nichols and Robert Roxby. University of Maine, Orono, Maine 04469

Molecular weight determinations of photosynthetic membrane proteins have relied almost exclusively on SDS-PAGE. This method is generally suspect for membrane proteins because anomalies in the interaction with detergent, compared to the soluble proteins ordinarily used as standards, may be expected. The method is particularly dubious for the chlorophyll-containing proteins of photosynthetic membranes, as these proteins continue to bind chlorophyll in detergents and give green bands in SDS gels. This indicates that some degree of native structure is retained and comparisons with the mobilities of soluble protein standards are therefore invalid. We have measured the molecular weight of CP-II, as the SDS complex, by the thermodynamically rigerous sedimentation equilibrium technique. The molecular weight determination was carried out by the method of Reynolds and Tanford (PNAS 73, 4467 (1970)). Sedimentation equilibrium and velocity measurements were made in H_2O containing buffers to determine the quantities $M(1-\bar{v}\rho)$ and the frictional coefficient. Sedimentation velocity measurements were then made over a range of solution densities, produced by substituting D_2O for H_2O, and extrapolated to a density at which the contribution of the detergent to the buoyant mass of the complex is nullified. At this point, the molecular weight of the protein alone may be estimated, employing reasonable assumptions about chlorophyll binding and $\bar{v}$, protein $\bar{v}$ and the effect of D_2O on sedimentation rates. The molecular weight obtained is 59000 daltons. The value estimated from SDS gels varies between 25000 and 30000 daltons. The anomalously high electrophoretic mobility may result from some combination of excess detergent binding and relatively compact shape of the CP-II complex compared to the standards.

T-AM-Pos98 DIFFERENTIAL SCANNING CALORIMETRY STUDY OF ASPARTOKINASE I-HOMOSERINE DEHYDROGENASE I. Leland P. Vickers. Department of Chemistry, Georgia State University, Atlanta, GA 30303.

Aspartokinase I-homoserine dehydrogenase I from E. coli is a bifunctional enzyme in the pathway of synthesis for 4 amino acids derived from aspartic acid. L-threonine is a feedback inhibitor of AKI-HSDI and stabilizes the tetrameric structure. A Hart Scientific high sensitivity differential scanning calorimeter was used to study the thermal denaturation of AKI-HSDI in the presence and absence of L-threonine. The buffer used contained 10 mM KPi, pH 7.6, 0.5 mM EDTA, 0.5 mM dithiothreitol, and 10 mM L-threonine (when used). AKI-HSDI in this buffer denatured in a single endotherm at 60°C. In the absence of threonine the denaturation occurred in two transitions at 51 and 58°C. When 0.15 M KCl was added to the above buffer, the denaturation in the presence of threonine included a large exothermic peak at 58°C, superimposed on the endothermic peak. In the absence of threonine two exothermic peaks were observed, at 42 and 56°C. The exothermic processes occurring at the higher ionic strength are presumably an aggregation of the denatured proteins. No transitions were observable in the down scans or subsequent scans up in temperature. The binding of the inhibitor threonine apparently causes the two major domains of AKI-HSDI to denature less independently. In all of these studies the protein concentration was approximately 1% and the scan rate was 30°C/hr. Supported by Research Corporation and ACS/PRF.

T-AM-Pos99 STRUCTURAL STUDIES OF MUTANT LAMBDA REPRESSOR PROTEINS: THE PHYSICAL BASES OF FOLDING AND FUNCTION. Michael A. Weiss,* Michael H. Hecht,** Hillary C.M. Nelson‡**Robert T. Sauer,** Dinshaw J. Patel,*** and Martin Karplus.*
*Department of Chemistry, Harvard University, Cambridge, MA. **Department of Biology, MIT, Cambridge, MA. ***Bell Laboratories, Murray Hill, New Jersey.

We have begun structural studies of mutant lambda repressor proteins which contain single amino-acid substitutions in the operator-binding domain. On the basis of the crystal structure, these substitutions may be classified as occurring in either surface or interior positions. The surface changes cluster in helix 2-turn-helix 3, the proposed DNA binding surface. The interior changes are widely distributed and often lead to temperature-sensitive phenotypes. We are using high-resolution proton magnetic resonance to study the tertiary structures of these altered proteins. Such studies give relationships between structure and function, as determined in vivo (host immunity to phage) and in vitro (operator-binding assay). The operator-binding domain, consisting only of five alpha helices, is an attractive model for protein folding. It thermally denatures at 52°C in a single calorimetric transition. We are using circular dichroism and scanning calorimetry to understand the thermodynamic basis of this transition in the wild-type and mutant domain. Thus the combined use of genetics and physical techniques may yield a detailed understanding of the relationships between levels of structure, folding and function.

T-AM-Pos100 SECONDARY STRUCTURE OF THE LAC CARRIER PROTEIN FROM ESCHERICHIA COLI. D. L. Foster, T. Goldkorn, N. Carrasco & H. R. Kaback (Intr. by J. P. Reeves), Roche Institute of Molecular Biology, Nutley, NJ 07110.

Circular dichroic studies on the lac carrier protein purified from Escherichia coli demonstrate that 85 ± 5% of the amino acid residues comprising this intrinsic membrane protein are arranged in helical secondary structures. Analysis of the hydropathic character of the primary sequence of the carrier indicates that the protein contains 12 hydrophobic segments with a mean length of 24 ± 4 residues per segment. Furthermore, these hydrophobic domains are punctuated by shorter hydrophilic regions which almost uniformly contain sequences predicted to form reverse turns. Taken together, the analyses suggest that the lac carrier protein is composed of 11-12 α-helical segments that traverse the membrane in a zig-zag manner as demonstrated for bacteriorhodopsin [Foster, D. L., Boublik, M. & Kaback, H. R. (1983) J. Biol. Chem., in press]. This model is currently being subjected to detailed investigation through the use of monoclonal antibodies that react with epitopes exposed on the inner and outer surfaces of the membrane. Monoclonal antibody affinity chromatography, together with reverse-phase high performance liquid chromatography, has proven successful in the isolation of fragments derived from the carrier by chemical and proteolytic cleavage. The chemical structure of the epitope-containing fragments is currently under investigation.

T-AM-Pos102 ACIDIC FORMS OF HORSE HEART FERRICYTOCHROME c: SPIN STATE, COORDINATION CONFIGURATION & CONFORMATION. Swatantar Kumar & Yash P. Myer, Inst. Hemoproteins, SUNYA, Albany, NY

Acidic forms of cytochrome c, the forms at pH 2 and 1, have been examined using resonance Raman (RR), circular dichroism (CD) and absorption spectroscopy as probes. Studies include the function of temperature and the addition of an anion. At pH 2 the absorption spectrum is typical of high-spin (HS) heme systems, with minimal temperature sensitivity between 30 and 4°. The CD spectrum is that of the unfolded protein with a loosened heme crevice. The RR spectrum at 30° is that of a HS hexacoordinated (HC) heme system, with indication of a minor component with features of a pentacoordinated (PC)-HS system. In contrast, the RR features at 4° are of LS-HC, HS-HC and HS-PC hemes. Lowering the pH to 1 at 30° generates a molecular form with RR features indicative of a HS-PC heme, which at 4°, becomes that of LS-HC heme. The CD spectrum at 30° is that of the unfolded protein, but at 4°, that of the partially folded molecule. The absorption spectrum changes from a typical HS form at 30° to a spectrum with 2:3::HS:LS forms. KCl at pH 2 and 30° causes changes similar to those observed in the RR spectrum upon lowering of temperature, but the changes are significantly different from those observed in absorption and CD measurements. The spectrum generated is that of a mixture of 1:1::HS:LS forms, the CD spectrum of the almost completely folded protein. KCl at pH 1 has little effect on the RR features, the absorption spectrum generated is distinct from either the low-temperature or the high-temperature forms, and the CD spectrum is that of the more folded protein. Based on RR parameters, in all the pH 2 forms, the iron is in the plane of the heme group, whereas the pH 1, 30° form and the pH 1, KCl form are pentacoordinated with iron out of plane by 0.38 ± 0.04 Å. (This work was supported by NSF Grant #PCM 792261.)

T-AM-Pos103 COMPUTER-GENERATED VAN DER WAALS MOLECULAR SURFACES, P.A. Bash, N. Pattabiraman, C. Huang, T.E. Ferrin and R. Langridge, Computer Graphics Laboratory, University of California, San Francisco, CA 94143.

Individual atomic surfaces are generated for a real-time interactive calligraphic color display system (E&S PS2). Each atomic surface consists of a set of dots distributed uniformly over a sphere, centered at the atom, whose radius is the van der Waals radius of the atom. Any overlap between atomic surfaces is removed. The algorithm for generating the surface is fast: 0.07 cpu seconds, on a VAX 11/750, per atom at a density of 5 dots per square angstrom. Not only is this much faster than earlier surface generators, but the surfaces maintain their proper representation during bond rotation, a great advantage in molecular modeling. For macromolecules, the surfaces of hidden atoms must be removed to provide an effective display. Our algorithm for locating hidden atoms is a two-step process. First the atomic coordinates are projected onto a discrete 3-D Cartesian grid. Next, each atom is checked for neighbors along the three orthogonal directions. Hidden atoms are defined as those atoms having neighbors on all six sides. This hidden-surface-removal algorithm is also quite fast: 0.02 cpu seconds per atom. The surfaces of these hidden atoms are removed because they provide little additional information and serve mainly to complicate the display. Any region of interest may be selected by checking the distances of atoms from a predefined set of coordinates. For example, the active site of an enzyme may be determined from the position of its associated substrate. The combination of van der Waals surfaces with bond rotation, hidden surface removal, and site selection is a powerful tool for real-time interactive molecular modeling. Supported by NIH RR-1081.

T-AM-Pos104 VISCOELASTICITY OF RIGID MACROMOLECULES WITH IRREGULAR SHAPES. William A. Wegener (Intr. by Robert M. Dowben), Muscle Research Laboratory, Baylor University Medical Center, 3500 Gaston Avenue, Dallas, Texas 75246.

A framework is presented to calculate the viscosity increment ν for a complex rigid body in a fluid undergoing steady or sinusoidal shearing. An arbitrary body is hydrodynamically described by six tensors to allow for rotational and translational motions coupled to each other and to the shear field. General ν expressions are obtained for dilute suspensions in the limit of overwhelming Brownian motion by balancing drag forces and torques with entropic forces and torques. In addition to a direct stress term there is also an entropic term of comparable magnitude arising from diffusive resistance to shear induced alignment. For sinusoidal shearing, ν is a complex number that generally exhibits five resonances at driving frequencies matching the $j = 2$ eigenvalues of the torque-free rotational diffusion equation. Resonance amplitudes depend on components of a body-fixed second rank tensor χ evaluated in the principal rotational axes. Special cases of symmetry are considered which reduce the number of contributing terms. Bead model expressions are derived in order to provide numerical treatments of complicated structures. Previous methods designed for simple particles are shown to generally overestimate ν for steady shearing by implicitly assuming fictictious forces and torques maintain body rotational and translational motions matching those of the local fluid. An algebraic criterion is found to locate the center of viscosity needed in these previous methods, although our treatment is independent of which point is used as an origin for calculations.

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