TH-POS-G1 INTERACTION OF NUCLEAR ACTOMYOSIN AND HISTONES. J. Birnbaum;* Intr. by P. Wachsberger. Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, PA 19174.

The contractile proteins actin and myosin have been identified in the nuclei of chick fibroblasts, chondrocytes, erythrocytes, and myoblasts. Actin and myosin are present in the mixture of proteins which are extracted when nuclei are exposed to 1 M KCl. Dialysis of the extract to low ionic strength (0.05 M KCl) results in the precipitation of a complex containing actin, myosin, and several other nucleoproteins, including histones. A complex is also precipitated when purified histones are mixed with muscle actomyosin, myosin, or fibroblast nuclear actomyosin and dialyzed to low ionic strength. Actin and histones precipitate immediately when mixed at low ionic strength. Histones, alone, or histones mixed with bovine serum albumin, do not precipitate when dialyzed to low ionic strength. A very slight precipitate forms when histones and actomyosin are mixed and maintained at high ionic strength. It is possible that contractile proteins may bind to histones in the course of cell fractionation, increasing the apparent concentration of such proteins in chromatin. However, control experiments indicate that contamination of nuclei with cytoplasmic proteins is insignificant. Thus, regulation of the interaction between histones and contractile nucleoproteins by changes in the ionic strength of the nucleus may be a factor in determining chromatin structure.

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TH-POS-G2 RECORDING OF A MEMBRANE POTENTIAL CHANGE IN THE CILIATE SPIROSTOMUM AMBIGUUM CORRELATED WITH MECHANICALLY STIMULATED RAPID BODY CONTRACTION. V.K-H. Chen, Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106

A membrane potential change has been recorded in the ciliate Spirostomum ambiguum correlated mechanically stimulated rapid body contraction. The rod shaped cell was made to span the oil gap between two electrolyte pools. One pool was held at virtual ground through a Ag/AgCl electrode connected to an operational amplifier. The other pool was connected thru a similar electrode in a suction pipette to an unity gain follower. Rapid body contraction was initiated by application of punctate mechanical stimuli. These stimuli were applied through a blunt glass rod moved by a piezoelectric drive. Only suprathreshold stimuli were used in this experiment. A biphasic potential was recorded between the two pools during rapid contraction of the Spirostomum spanning the oil gap. The biphasic waveform became essentially monophasic if there was a good oil seal around the cell. The waveform was no longer detectable when the cell became habituated to the mechanical stimuli.

The biphasic potential waveform can be interpreted as the result of membrane potential changes propagating along the cell. The transformation of the biphasic waveform to an essentially monophasic waveform is then due to blocking of the propagated membrane potential by the oil gap. The recording of this contraction potential allows the excitation-contraction coupling in this non-muscle cell to be studied. Microfilaments are known to be involved in the contractile apparatus of this cell.

TH-POS-G3 CHARACTERIZATION AND USE OF FLUORESCEIN LABELLED MYOSIN S-1 AS A PROBE FOR ACTIN IN NON-MUSCLE CELLS. J.A. Schloss* and R.D. Goldman, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pa. 15213.

Rabbit skeletal muscle myosin subfragment-1 (S-1) was conjugated with fluorescein isothiocyanate and separated into fractions by ion exchange chromatography. The fractions varied in their average fluorochrome-to-protein ratios. The specific activity (actin activation of ATPase in 2.5mM $Mg^{++}$) of these conjugate (FS-1) fractions decreased in inverse relation to the number of bound fluorescein molecules. After 6 hour conjugation at pH 7.5 to 7.8, three FS-1 fractions retained 67, 55 and 37% of their ATPase activities; they contained an average of 2.1, 2.9 and 4.0 fluorochromes per S-1, respectively. Fluorescence microscopy of rat embryo cells, glycerinated and incubated with FS-1, revealed fluorescent fibers which corresponded to phase dense stress fibers. The staining pattern obtained from the three fractions was identical. Nuclear and nucleolar fluorescence were greatly decreased relative to that seen in cells stained with FS-1 prepared by more conventional fractionation on G-25 Sephadex. Competition of FS-1 with S-1 completely eliminated fiber fluorescence, but had little effect on nuclear fluorescence. FS-1 formed arrowheads with rabbit skeletal actin and decorated microfilaments in cultured cells. Pyrophosphate and ATP did not remove FS-1. This appeared to be related to a deterioration in cell structure and solubilization of cell proteins. FS-1 staining of mitotic Pt K1 cells revealed poles, kinetochores and spindle fibers. Ultrastructural correlates of these observations will also be presented.

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**TH-POS-G4** DEFINITION OF THE DISTANCE VARIABLE IN MATHEMATICAL MODELS OF MUSCLE CONTRACTION
Stephen J. Koons, Department of Biophysical Sciences and College of Mathematical Sciences, State University of New York at Buffalo, 4234 Ridge Lea Rd., Buffalo, New York 14226

The definition of a distance variable is central to any kinetic model of muscle contraction. Usually the distance x is defined as the distance, along the filament axis, from the equilibrium position of the myosin cross-bridge to the nearest actin binding site. Cross-bridges are distributed uniformly in x. Cross-bridge free energies and forces are functions of x, as are rate constants for transitions among biochemical states. This definition has the disadvantage that all information on the rate of attachment as a function of the distance variable must be included in the rate constant alone. Also, allowed values of x depend upon the state of the cross-bridge; for attached cross-bridges the limits of x depend upon the myosin structure, as reflected in the free energy curve, while for detached cross-bridges the limits are specified formally by the separation of actin binding sites. These inconsistencies may be corrected if a new distance u is defined as the distance, along the filament axis, from the equilibrium position to the myosin cross-bridge. Cross-bridges are not uniform in u, but the free energies are now of the same form for all states. Note that x and u are identical for attached cross-bridges. Detached cross-bridges are assumed to be at equilibrium with respect to position. The population density of these cross-bridges is proportional to the exponential of the negative free energy, and becomes an important factor in the rate of attachment. Since the free energy depends primarily upon the myosin structure and the nature of the force generating element, the use of u instead of x enhances the correspondence between muscle structure and theoretical models.

**TH-POS-G5** PHLORETIN-INDUCED CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM FRAGMENTS. K. W. Anderson, Laboratory of Physiology and Biophysics, University of the Pacific, San Francisco, California 94115.

Phloretin, a strongly dipolar hydrophobic molecule, causes calcium release from actively-loaded vesicles of sarcoplasmic reticulum (SR) isolated from rabbit white skeletal muscle. One minute after initiating calcium uptake with ATP, phloretin (in 95% ethanol) is added and the extent of calcium release determined. At pH 6.8 and 0.1 mM phloretin, ~20% of the accumulated calcium is released; complete release is induced by 1mM phloretin. Increasing the temperature from 25 to 37°C does not affect the magnitude of the release. When the vesicles are incubated with phloretin before initiating active uptake, both the rate and the extent of calcium accumulation are decreased. Phloretin also inhibits the ATPase activity of SR, with half-inhibition at 0.5 mM phloretin. Increasing the buffered free calcium concentration outside the vesicles enhances the phloretin-induced calcium release. At a fixed total calcium concentration of 0.1 mM, the magnitude of the release decreases linearly with pH over the range 5.5 to 8.0. Phlorizin, a non-dipolar molecule sterically similar to phloretin, causes similar calcium release from SR vesicles, but a concentration 40 times the phloretin concentration is required. It is suggested that phloretin-mediated reduction of the SR membrane dipole potential may be responsible for the observed effects of calcium release.

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**TH-POS-G6** A VOLTAGE CLAMP ANALYSIS OF THE ACTIVE INWARD CURRENTS IN THE FLIGHT MUSCLE OF THE FLY, *MUSCA DOMESTICA*. J. B. Patlak, Department of Physiology, U.C.L.A. School of Medicine, Los Angeles, California 90024.

Voltage clamp analysis of the dorsal-longitudinal flight muscle in the common housefly, *Musca domestica*, has provided information about the inward currents responsible for the $Ca^{++}$ spike and the $Na^{+}$ plateau which were previously reported by this author in a similar preparation (J. Comp. Physiol., 107: 1, 1976). A portion of this cell was voltage clamped in situ with a standard feedback circuit. This 400 μm cell lies directly beneath the cuticle of the posterior thorax, and the end of the cell attaches to the cuticle in a different part of the thorax. The cuticle over the side of the cell was removed under ringer solution and $V_m$ was measured differentially between two microelectrodes. Current was passed into the end of the cell through a 30 μm hole in the cuticle which was isolated from the bath by an air gap. This greatly reduced the access resistance compared to microelectrode current injection. Measurement of membrane current was made from a small (60 μm diameter) area of the membrane using a Neher & Lux type patch electrode (Pflug. Arch. 311: 272, 1969) placed near the $V_m$ measuring electrodes. Patch potentials were held at the reference level. Inward $Ca^{++}$ currents were measured in the presence of TEA, which blocked the temporally overlaping delayed rectification. These potential dependent $Ca^{++}$ currents were similar to those seen in crustacean muscle by various investigators. In addition, a potential dependent $Na^{+}$ current with very slow kinetics is seen. This has not been reported in other preparations. Evidence is presented for interaction between these two ions in this system. (This work was supported by USPHS Grant NS09012 to Dr. Hagiwara and Training Grant GM 448).

TH-POS-G7 SOLVENT ACCESSIBILITY AND EFFECTS OF ACTIN-MYOSIN INTERACTIONS ON THE BOUND NUCLEOTIDE OF ACTIN, INVESTIGATED WITH THE FLUORESCENCE OF 1,$N^6$-ETHENOADENOSINE NUCLEOTIDES. S. C. Harvey and H. C. Cheung, Biophysics Section, Department of Biomathematics, University of Alabama in Birmingham, Birmingham, Alabama 35294

1,$N^6$-ethenoadenosine nucleotides (ε-ATP and ε-ADP) have been used as fluorescent probes of the nucleotide binding site of actin. The fluorescence of ε-ATP bound to G-actin is not quenched by iodide ion, tryptophan, methionine, or cysteine, although all of these are collisional quenchers of ε-ATP free in solution. Consequently, the fluorescent base is bound in a region of the protein which is inaccessible to collisions with these reagents. Since the fluorescence of free ε-ATP is also quenched by water, the long lifetime of the bound nucleotide (36 nsec vs 27 nsec for ε-ATP in solution) results from the fact that the base is inaccessible to water. Measurements of both the fluorescence lifetime and the polarization of fluorescence of ε-ADP bound to F-actin show that the actin filament undergoes cooperative conformational changes when either heavy meromyosin (HMM) or myosin subfragment-1 (S1) binds to the filament. On a molar basis, S1 is just as effective as HMM in producing these effects, so the second head of HMM apparently does not have a role in causing the conformational changes that are being detected.

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TH-POS-G8 PROTON MAGNETIC RESONANCE STUDIES OF RABBIT SKELETAL TROPOMYOSIN. B.F.P. Edwards* and B. D. Sykes, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Renatured αα-tropomyosin from rabbit skeletal muscle has been studied using proton nuclear magnetic resonance at 270 MHz. Each chain of the dimer has one histidine residue near the centre (HIS 153) and one near the C-terminal end (HIS 276) of the coiled-coil structure (Stone, D. et al. in Proc. IX FEBS Meeting, 1975, Proteins of Contractile Systems). Our spectra support the finding that the two chains are in register, related by a dyad. We have assigned the resonances of the histidines using carboxypeptidase digestion and have determined their acid dissociation constants. The pair of central histidines titrate in a cooperative manner giving Hill coefficients approaching 2.0 under certain conditions. The resonances of the two HIS 276 residues, which are thought to be in the overlap region of polymerized tropomyosin, are perturbed by the polymerization.

TH-POS-G9 PRELIMINARY STUDIES OF THICK FILAMENTS RECONSTITUTED FROM LIMULUS MYOSIN AND PARAMYOSIN. R.J.C. Levine, F.A. Pepe, M.J. Elfvin and V. Sawyna. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, Pa. 19129 and Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19174.

In preliminary experiments, thick filaments were reconstituted from purified Limulus myosin (LM) and paramyosin (LPM), both alone and mixed at several different molecular ratios (PM:M): 0.5, similar to the ratio in Limulus skeletal muscle; 2.0, similar to that in Mytilus ABRM; and 0.07, similar to that in Aequipecten striated adductor (Levine, et al, J. Cell Biol. 71:273,'76). The proteins were aggregated by dilution of the medium from 0.6 M KCl in 10mM imidazole, pH 7.0 to 0.15M KCl in 10mM imidazole, with constant stirring in the cold, according to Pepe's two step procedure.

Thus far, we find that filaments formed from LM alone or together with LPM almost always have central bare zones and are distinctly bipolar. The length of filaments reconstituted in this way from LM alone or mixtures having PM:M ratios of 0.07 or 0.5 is about 2.5μm. Longer filaments form at a PM:M ratio of 2.0. Crossbridge structure is most clearly defined in the presence of 5mM ATP and 5mM $Mg^{++}$. Occasionally we see paracrystals, having 14.5 nm periodicity, similar to structures which have been reported in intact Limulus muscle (Levine, et al, J. Cell Biol. 57: 591,'73). LPM alone forms aperiodic fibrillar meshworks. Studies of the effect of divalent cations on the organization of such reconstituted filaments and optical diffraction analyses of filament substructure are underway.

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**TH-POS-G10** STOPPED-FLOW STUDY OF THE RATE OF CALCIUM BINDING BY EGTA. P.D. Smith, R.L. Berger, R. J. Podolsky, Laboratory of Technical Development, NHLBI, Laboratory of Physical Biology, NIAMDD, Bethesda, Md. 20014, and G. Czerlinski, Northwestern University, Chicago, Illinois 60611

Ethyleneglycol bis(aminoethylether)tetraacetic acid (EGTA) is a well-known chelating agent for divalent cations. In particular EGTA has a preferential affinity for calcium over magnesium (stability constants 11.0 and 5.2, respectively) and has found use as a calcium buffer in biological experiments. A study of the rate of calcium binding by EGTA was carried out using a rapid mixing optical stopped-flow apparatus (1), with a dead time of 200 μs. Concentrations of calcium chloride and EGTA were varied between 0.1 mM and 1mM, at 8° and 25°C. The solutions were buffered by 10 mM cacodylic acid at pH 7.00 before mixing, and the experiments were carried out in the presence of 100 mM KCl. The reaction progress was observed by monitoring the optical density change at 620 nm using 0.1 mM bromthymol blue as an indicator. $2H^+$ ions are released for each molecule of Ca-EGTA complex formed. The data were collected on a Biomation 802 A/D converter and analyzed by the finite element analysis simulation technique (2) for the $H^+$ ion released as a function of time in terms of the buffer system and the optical density change of the indicator. Reaction rates at pH 7.0 of $2 \times 10^6$ $M^{-1}$ $s^{-1}$ (25°C) and $5 \times 10^5$ $M^{-1}$ $s^{-1}$ (8°C) were obtained. Temperature jump experiments lead to similar results

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2. N. Davids and R. L. Berger Com. of Amer. Comp. Mach. Assn. 7, 547 1969.

**TH-POS-G11** CORRELATION OF ELECTRICAL COUPLING WITH MORPHOLOGY IN MYOCARDIAL CELLS IN CULTURE. R.B. Robinson, K. Hermsmeyer, and W. Larsen*, Dept. of Pharmacology and Dept. of Anatomy, The Cardiovascular Center, Univ. of Iowa, Iowa City, Iowa 52242

The extent of electrical coupling of myocardial cells in culture was studied and correlated with morphology. Ventricular cells from 13-day embryonic chicks or 4-day neonatal rats were trypsin-dispersed and plated onto coverslips, resulting in networks or sheets of spontaneously contracting cells. For experiments, coverslips were transferred to a constant suffusion chamber (37°C) and observed by Nomarski optics. Two microelectrodes, each in a bridge configuration, were used to record membrane potential simultaneously from interconnected cells. Current (< 3 nA) could be passed through either electrode and hyper- or depolarization recorded at both points only if both electrodes were intracellular. Coupling ratios were computed as a function of inter-electrode distance. For some experiments, electrodes were filled with the fluorescent dye, sodium fluoresceinate, which could be iontophoretically injected into the cell to delineate its borders (this dye passes from cell to cell very slowly). Preliminary data suggest that the rat ventricle cells in culture are much more strongly electrically coupled than the chick ventricle cells, although cells from both rat and chick demonstrated synchronous activity (including synchronous subthreshold oscillations). In addition, freeze-fracture studies of the cultures show the chick ventricle to have small gap junctions, often in linear configuration. Rat ventricle demonstrated larger gap junctional particle aggregates, providing a possible morphological correlation with the electrical data. (Supported by grant HL 16328 from the National Institutes of Health and GRE support from the University of Iowa College of Medicine)

**TH-POS-G12** ADENOSINE 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE PHOSPHORYLATION OF A BOVINE CARDIAC ACTIN COMPLEX. Gary Bailin, Depts. of Physiology and Biophysics, and Medicine, Mt. Sinai School of Medicine of CUNY, New York, New York 10029

Phosphorylation of a bovine cardiac actin-tropomyosin-troponin complex was stimulated when the complex was incubated with [$\gamma$-$^{32}$P]ATP, $Mg^{2+}$, adenosine 3':5'-monophosphate (cyclic AMP) and bovine cardiac cyclic AMP-dependent protein kinase (protein kinase). No significant phosphoprotein was found when the actin complex was incubated with cyclic AMP but without protein kinase. Maximal incorporation of 0.22 mole [$^{32}$P]phosphate per mole of actin complex occurred at pH 8.0. Approx. 81% of the [$^{32}$P]phosphate bound to the complex was identified as phosphoserine and phosphothreonine. Polyacrylamide gel electrophoresis in SDS showed that 55% of the [$^{32}$P]phosphate was associated with the inhibitory component of troponin (Tn-I) and 24% was associated with the tropomyosin binding component of troponin. Phosphorylation of Tn-I in the actin complex was inhibited 30% when the $Ca^{2+}$ concentration was increased from $10^{-7}$ to $5 \times 10^{-5}$ M. No change in the distribution of phosphoamino acids was seen at low or high $Ca^{2+}$. Half maximal $Ca^{2+}$ activation of the ATPase activity of reconstituted actomyosins (AM) made with the [$^{32}$P]phosphorylated actin complex and cardiac myosin was shifted to higher $Ca^{2+}$ concentration when compared with a reconstituted AM made with an actin complex incubated without protein kinase. An increase in the [$^{32}$P]phosphate content of the actin complex decreased the sensitivity of the reconstituted AM to $Ca^{2+}$. Double reciprocal plots of $Ca^{2+}$-dependent ATPase of reconstituted AM made with the [$^{32}$P]phosphorylated actin complexes were curvilinear and indicated some degree of altered cooperativity when compared to a control reconstituted AM. These findings suggest the possible involvement of cyclic AMP-dependent protein kinase phosphorylation of troponin in modulating interactions between $Ca^{2+}$ and cardiac muscle AM. Supported by grants from MDA and NIH No. AA-00316.

**TH-POS-G13** CHANGES IN LENGTH, DIAMETER AND VOLUME OF THE THICK FILAMENTS OF LIMULUS STRIATED MUSCLE DURING SARCOMERE SHORTENING. M.M. Dewey, D. Colflesh, B. Walcott. and R.J.C. Levine, Anatomical Sciences, SUNY at Stony Brook, Stony Brook, N.Y. 11794 and Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, Pa. 19129

We have previously reported thick filament length changes (shortening) in Limulus striated muscle (Dewey et al. J. Cell Biol. 58:574-593, 1973). Here we report changes in diameter and volume of the thick filaments during shortening of the sarcomere. Muscles were fixed at various lengths (tension developed during fixation at each length) and final sarcomere length was determined by laser diffraction (Dewey et al. Biophys. Abst. Feb. 1972). Transverse sections were photographed (X 50,000) and thick and thin filaments were traced from projected negatives (X 1,000,000). Thick filament profiles were always elliptical. These elliptical profiles were not due to the plane of section since the angle of the major axis of the ellipse was randomly oriented in each section. As the sarcomere shortened from 7.4 um to 4.4 um, the thick filaments increased in diameter uniformly in both directions (x=246 A, y=220 $A_2$ at 7.4 um to x=366 A, y=293 A at 4.4 um). The calculated area changed from 42.5 x $10^6$ $A^2$ to 84.9 x $10^6$ $A^2$. Using the filament lengths previously determined, the filament volume changed from 20.4 x $10^4$ $A^3$ to 27.2 x $10^4$ $A^3$. A model is presented which predicts an increase in volume of the filament with decreased length if the filament 1. is composed of subunits consisting of $\alpha$ helical portion of the constituent molecules and 2. as each subunit is intercalated into the filament, it retains constant length, diameter and intersubfilament spacing.

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**TH-POS-G14** EFFECTS OF ENHANCED INTRACELLULAR $Ca^{2+}$ RELEASE ON THE LENGTH-TENSION RELATION OF ISOLATED SKELETAL MUSCLE FIBERS. J.R. Lopez*, L.A. Wanek*, and S.R. Taylor, Department of Pharmacology, Mayo Graduate School of Medicine, Rochester, Minnesota 55901

Reduced release of intracellular $Ca^{2+}$ may normally be one of the factors that limit the ability of muscle to develop active tension at short or long lengths. We tested the influence of this factor by measuring the length-tension relation of muscle fibers bathed in solutions containing $Zn^{2+}$, which apparently acts at sites in contact with the external solution to enhance the release of activating $Ca^{2+}$ inside a contracting muscle cell (Lopez et al., 1976, J. Gen. Physiol. 68:11a). Single fibers were isolated from tibialis anterior and semitendinosus muscles of the frog Rana temporaria, injected with the photoprotein aequorin which luminesces in the presence of $Ca^{2+}$, and electrically stimulated every 5 min at 15°C to elicit a twitch and tetanus. Light emission and contractile force were monitored at several striation spacings between 1.6 and 2.8 μm, before and after $Zn^{2+}$ was added to the bath. At all muscle lengths, $Zn^{2+}$ increased the peak intensity (and total amount) of light emitted during contraction by at least 3-fold. Tension was increased most markedly at the shortest muscle lengths. For example, peak tetanic tension increased 1.6 fold at the shortest length and increased 1.1 fold at the plateau of the length-tension relation. At longer lengths peak tetanic tension was not significantly increased despite the apparent increase in $Ca^{2+}$ release, which suggests that activation was maximal in this range. These results support the view that the degree of overlap between the contractile filaments is the major determinant of the length-tension relation at long muscle lengths, but that decreased activation is a major factor at short muscle lengths. (Supported by the CONICIT of Venezuela, NS 10327 from the USPHS, and by an Established Investigatorship from the American Heart Association.)

**TH-POS-G15** DIRECT VISUALIZATION OF THE NETWORK NATURE OF THE T-SYSTEM OF FROG SKELETAL MUSCLE AND ITS SURFACE CONNECTIONS BY HIGH VOLTAGE ELECTRON MICROSCOPY. Lee D. Peachey and Clara Franzini-Armstrong, Department of Biology, University of Pennsylvania, Philadelphia, PA. 19174

A somewhat idealized view of the T-system (transverse tubular system) of frog skeletal muscle fibers has been obtained by electron microscopy using thin sections. High voltage electron microscopy (HVEM) combined with selective staining of the T-system, has extended our three-dimensional knowledge of T-system structure to include a spiral arrangement (Peachey and Eisenberg, Biophys. J. 1975, 15, 253a) and longitudinal extensions of the T-system in the region of dislocations in the band structure (Peachey, J. Cell Biol. 1976, 70, 356a). Lanthanum nitrate is a more intense T-system selective stain* than stains used in the earlier studies. This has allowed the examination of very thick (3μm or greater) slices of muscle tissue by HVEM, and has facilitated the attainment of superior stereoscopic views of surface connections and other features of the T-system. Among the findings that have come out of this work are: 1) Most surface connections of the T-system are into surface caveolae, often into more than one, 2) many surface connections are via a very narrow tubule into the caveola, and thence to the true fiber surface via a narrow caveolar neck, 3) Groups of caveolae can be connected together in rows by a fine tubule (not part of the T-system), usually at the level of the Z-lines, 4) Openings of the T-system are closer together transversely than were the sensitive spots in local activation experiments of Huxley and Taylor (J. Physiol. 1958, 144, 426), and 5) Longitudinally-oriented T-tubules are common just below the fiber surface. (Supported by grants from NIH(HL 15835) and the MDA (Henry M. Watts Neuromuscular Disease Center).

*Our appreciation to C. Peracchia for one of the best embeddings with this stain.

TH-POS-G16 LENGTH DEPENDENCE OF INITIAL HEAT IN CARDIAC MUSCLE. L.A. Mulieri and N.R. Alpert. Dept. Physiology & Biophysics, University of Vermont, Burlington, Vt. 05401

Initial heat was measured in rabbit right ventricular papillary muscles under isometric conditions (21 to 25°C). Single muscles were mounted on planar, Hill-type thermopiles made with vacuum deposited bismuth-antimony thermocouples (Mulieri, Luhr, Trefry and Alpert, The Physiologist, 19, 307, 1976) and stimulated at a rate of 0.2 Hz with 2 msec rectangular pulses applied via a punctate electrode at each end of the muscle. Initial heat in a single twitch was estimated by measuring the temperature change accompanying the mechanical response. A mean value of 1.68±0.16 m°C was obtained at optimum length ($\ell_o$) in 4 muscles ranging in weight from 1.2 to 4.7 mg and developing a peak twitch tension ($P_{\ell_o}$) of 6.40± 0.17 g/mm$^2$(mean ± SEM). The relation between initial heat ($H_\ell$) and isometric twitch tension ($P_\ell$) was examined at muscle lengths ($\ell$) ranging from 0.65$\ell_o$, where $P_\ell$ was reduced 98%, to 1.15$\ell_o$ where $P_\ell$ was reduced 41%. Below $\ell_o$ the initial heat decreased as twitch tension decreased. Although the average relation was somewhat curvilinear ($H_\ell/H_{\ell_o}$=0.07 + 0.44 $P_\ell/P_{\ell_o}$ + 0.44 $[P_\ell/P_{\ell_o}]^2$, RMS error = 0.045) the data suggest an approximately constant amount of heat is generated per unit of twitch tension. In contrast, stretched muscle displayed an inverse relation between initial heat and twitch tension. Initial heat per unit of twitch tension nearly doubled over the range of lengths from $\ell_o$ to 1.15$\ell_o$. This difference between heat per unit of tension in stretched as compared to shortened muscle may indicate length dependent changes in excitation-contraction coupling and/or in the efficiency of tension generation. Supported by American Heart Assoc. Grant-in-Aid #75 822 and U.S.P.H.S. Grant #PHS R01 17592-01 and T01-05707.

TH-POS-G17 SHARPER DIFFRACTION PATTERNS FROM HEART MUSCLE WITH INFRARED LIGHT. J.W. Krueger, Depts. of Medicine and Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Light diffraction methods have been used to study the role of myofilament sliding in mammalian cardiac muscle contraction, but sharp diffraction patterns have been obtained only in very thin (<200 μm) preparations, thereby limiting past studies to strips of rat heart muscle. In view of the ability of infrared light to penetrate cardiac tissue (L. Massopust, Anat. Rec. 61: 71, 1934) it has been used to form diffraction patterns from rabbit and rat right ventricular papillary muscles 300 to 750 μm thick. A gallium-arsenide pulsed laser diode transmitter (904 nm peak emission, 3.5 nm spectral bandwidth, 2.9 mW average power) was used as a source of light in the near-infrared range. Diffracted light was collected with a 50X, N.A. 1.0 water immersion objective lens (E. Leitz). The resulting first order diffracted light intensity distribution was narrower than that obtained with a helium neon laser ($\lambda$=632.8 nm, 5 mW power). The relative improvement in spectral sharpness with infrared was most pronounced in the thicker muscles; in many, no first order spectra could be detected with the shorter wavelength. Small peaks often occurred on the first order diffracted light late during the interval between contractions in rat heart muscle. Their positions were not stationary. Thus, despite uncertainties about inference of the sarcomere length dispersion by diffractometry, multiple, discrete sarcomere populations could be resolved with the infrared illumination. The infrared diffraction technique permits direct and quantitative comparison of sarcomere dynamics in the relatively thicker muscle preparations from species with postulated differences in contractile activation and control.

TH-POS-G18 THE EFFECT OF SARCOMERE LENGTH ON THE BINDING OF CALCIUM TO GLYCERINATED MUSCLE FIBERS. F. Fuchs, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

A double isotope technique was used to measure the binding of $Ca^{2+}$, under rigor conditions, to thin rabbit psoas bundles extracted with 1% Triton X-100 and 50% glycerol. Free $Ca^{2+}$ concentration was controlled by EGTA buffers and sarcomere length was controlled by pre-stretch (or release) of muscle bundles prior to extraction. These experiments were designed to test the effect of rigor complex formation, determined by the degree of filament overlap, on $Ca^{2+}$-receptor affinity in the intact filament lattice. In the presence of 5mM $MgCl_2$ (no ATP) reduction of filament overlap was associated with reduced binding of $Ca^{2+}$ over the entire range of free $Ca^{2+}$ concentrations ($10^{-7}$-$10^{-5}$M). With maximum filament overlap (sarcomere length 2.0 - 2.2 μm) the maximum bound $Ca^{2+}$ was equivalent to 4 moles $Ca^{2+}$/mole troponin. Scatchard and Hill plots showed significant positive interaction between binding sites. With no filament overlap ( sarcomere length > 3.8 μm) the maximum bound $Ca^{2+}$ was equivalent to 3 moles $Ca^{2+}$/mole troponin and graphical analysis indicated a single class of non-interacting sites. The data indicate that 1) in the intact filament lattice one of the $Ca^{2+}$-binding sites is present only when cross-bridge attachments are formed and 2) the formation of such attachments converts the binding sites from a non-interactive to a positively interacting system.

Supported by grants from the NIH (AM 10551) and the Muscular Dystrophy Association.

TH-POS-G19 BIOLOGICAL CROSS-REACTIVITY OF SKELETAL MUSCLE TROPONIN-C (TnC) AND TESTIS PHOSPHODIESTERASE ACTIVATOR PROTEIN. J. Dedman*, J.D. Potter, R.L. Jackson*, and A.R. Means*, Depts. Cell Biol. and Cell Biophys., Baylor Coll. Med., Houston, TX 77030 (Spon. C.L. Seidel)

Phosphodiesterase activator protein (AP) has been purified from rat testis and the properties compared with that of rabbit skeletal muscle TnC. AP migrates slightly faster than TnC on 15% polyacrylamide gels in the presence of SDS with an estimated molecular weight of ~ 17,000. The molecular weight of AP calculated from the amino acid composition is 16,900. AP contains 1 histidine, 2 tyrosines, 2 prolines but no cysteine or tryptophan. Like TnC, AP binds 4 moles of calcium/mole. In light of these similarities between AP and TnC it was of interest to compare their biological cross-reactivity. To test for complex formation between AP, rabbit skeletal muscle troponin-T (TnT) and troponin-I (TnI), the three proteins were mixed together in a 1:1:1 molar ratio in the presence of 1M KCl. After dialysis against 0.1M KCl no precipitate was observed, indicating complex formation. This soluble hybrid complex conferred calcium sensitivity on skeletal muscle actomyosin ATPase. In the absence of calcium, the ATPase inhibition was greater than with an equivalent amount of native troponin complex (Tn). In the presence of calcium, the ATPase was stimulated, although it was lower than with Tn. For activation of phosphodiesterase (PDE), the assay required a 500-fold greater concentration of TnC than of AP for full stimulation and this activity was calcium-independent, in contrast to AP activation of PDE. As a control, similar concentrations of carp parvalbumin had no effect on the PDE activity. Thus, although these proteins are similar in many ways, it is clear that functionally they are not totally interchangeable. (Supported by grants from NIH (HD 07503), the American Heart Association and the Texas Affiliate, American Heart Association. J. Potter and R.L. Jackson are Established Investigators of the American Heart Association.)

TH-POS-G20 CHLORIDE CONDUCTANCE AND DIAZACHOLESTEROL INDUCED MYOTONIA IN RAT MUSCLE. S.H. Bryant and S.L. Hershneck*, Dept. Pharmacology and Therapeutics, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267

It has been reported that muscles from rats made myotonic by several weeks treatment with diazacholesterol have a decreased chloride conductance which was suggested to be the basis of the myotonic behavior (Rüdel and Senges, Naunyn-Schmiedeberg's Arch. Pharmacol. 274:337-347,1972). Using a new method described by Dromgoole et al.(Biochem. Med. 13:307-311,1975) we treated male rats with large single oral doses of diazacholesterol (200 mg/Kg) which caused the development of myotonia without the usual ill health seen in the chronically treated animal. We were interested then in whether the myotonia was associated with a lowered chloride conductance under these circumstances. Rat extensor digitorum longus (EDL) muscles were studied in vitro at 23° C in bicarbonate or tris buffered physiological solution. We observed fiber excitability, membrane potentials and cable parameters using two intracellular microelectrodes, one for passing current and the other for recording membrane potentials. From the membrane resistances in normal and chloride-free (methylsulfate) solutions we calculated the component conductances. All of these measurements were made in control and in treated EDL fibers at 3, 5, 9, 11 and 16 days following the drug. Although the chloride conductance began to decrease at 16 days (down 38%) evidence of myotonia (EMG-signs, repetitive firing, decreased threshold) was recorded at 5, 9 and 11 days when chloride and potassium conductances were in the normal range. The results suggest that there is a membrane change, possibly a shift in sodium activation kinetics, in addition to the block of the chloride channel in diazacholesterol myotonia. (Supported by NIH Grant NS03178)

TH-POS-G21 KINETIC STUDIES OF THE MECHANISM OF DEPHOSPHORYLATION OF SARCOPLASMIC RETICULUM ADENOSINE TRIPHOSPHATASE. J.P. Froehlich,* and F.J. Schenck*. Introduced by G.L. Eichhorn. Laboratory of Molecular Aging, National Institute on Aging, National Institutes of Health, Baltimore, Md. 21224

Rapid chemical quenching was used to study the effects resulting from the simultaneous addition of 4 mM EGTA, 1 mM $MgCl_2$, and different concentrations of [$\gamma$-$^{32}$P] ATP to a suspension of sarcoplasmic reticulum vesicles preequilibrated with 20 $\mu$M $Ca^{2+}$. The acid stable phosphoenzyme (E-P) formed under these conditions arises only from $Ca^{2+}$ which is initially bound to the enzyme since EGTA rapidly reduces the level of free $Ca^{2+}$ to below $10^{-8}$ M. The presence of the chelating agent reduced the amplitude of the phosphoenzyme burst and stimulated E-P decay by preventing rephosphorylation of the enzyme. The disappearance of phosphoenzyme, which was accompanied by the simultaneous release of $P_i$, followed a simple first order decay process. $P_i$ production attained levels equal to the maximum size of the hydrolytic burst observed under optimal labelling conditions. In contrast to the behavior when $Ca^{2+}$ is saturating, the rate constant governing E-P decay was insensitive to variation in ATP concentration over a fairly wide range. Disappearance of the effects of substrate activation in the presence of EGTA suggests that $Ca^{2+}$ is required for expression of the allosteric ATP binding site which regulates dephosphorylation. The results are discussed in terms of a flip-flop mechanism in which phosphorylation in the presence of EGTA involves only one-half of the catalytic sites. The effect of $Ca^{2+}$ on the rate of dephosphorylation is linked to activation of phosphorylation in the neighboring catalytic unit.

**TH-POS-H1** SIZE LIMIT OF MOLECULES PERMEATING JUNCTIONAL MEMBRANE. I. Simpson[‡] and B. Rose, Dept. Physiology and Biophysics, University of Miami School of Medicine, Miami, Fla. 33152

The permeability of junctional membrane in Chironomus salivary gland was probed with amino acids and synthetic or natural peptides labelled with fluorescent dyes[1]. Molecules up to 1200 dalton passed through the junction. Molecules > 1900 dalton did not pass. The largest of the permeating molecules was LRB $(Leu)_3(Glu)_2OH$, 1158 dalton. The smallest of the NON-permeating molecules was FITC-Fibrinopeptide A, 1926 dalton. This passage failure may reflect the normal size limit for junctional membrane permeation. From this size limit and the molecular dimensions of the labelled compounds a diameter of 10-14Å is estimated for the junctional membrane channels. The size limit for normally permeating molecules (< 1200 dalton) could be reduced in a graded fashion with varying cytoplasmic $[Ca^{++}]$.

[1]Fluorescein isothiocyanate (FITC), Lissamine rhodamine B (LRB) and Dansylchloride. All labelled compounds carried at least one negative charge.

**TH-POS-H2** THE INFLUENCE OF LANTHANUM ON THE NEXUS OF ABRM, R. Kensler*, P. Brink* and M.M. Dewey, Anatomical Sciences, SUNY at Stony Brook, Stony Brook, N. Y. 11794.

The first evidence for nexuses in ABRM of Mytilus was described in thin section by Twarog et al. (J. Gen. Physiol. 61:207-221, 1973). Subsequently we have found that when the ABRM was exposed to seawater containing ionic lanthanum and then fixed, the nexuses were stained such that the extracellular leaflets as well as the normally electron lucient region became electron opaque. Freeze-fracture replicas made from ABRM, fixed and unfixed, showed nexuses with particles in the PF face and pits in the EF face in an hexagonal array. Optical diffraction showed nearest neighbor packing with no long range order. Tissues exposed to a lanthanum seawater rinse (30-180 min) before glyceration or fixation showed tight packing of the EF face pits and optical diffraction of these images showed nearest neighbor spacing with long range order. The PF particles appeared as small aggregates and showed disorder. The most significant result of long lanthanum exposure is the frequency of nexuses. Lanthanum appears to severely reduce the number of nexuses found suggesting that in time lanthanum disrupts the nexuses completely. Lanthanum (5-30 mM) containing seawater was prepared by using a Tris-maleate, Tris buffer system (pH=6.8). Using bicarbonate buffer, pH 6.5, Twarog (personal communication) observed only a slight depolarization in the resting potential. Nexuses of tissues exposed to pH 6.8 seawater for as long as 3 hr (no La) showed no disruption of nexuses and the frequency of nexuses was normal (90 min). Since the nexuses are disrupted and their frequency is reduced when exposed to lanthanum, it is concluded that lanthanum uncouples adjacent cells (see R. Brink, M. M. Dewey and D. Colflesh this meeting)

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**TH-POS-H3** NEXAL MEMBRANE PERMEABILITY TO VARIOUS FLUORESCENT DYES AND THE INFLUENCE OF LANTHANUM. P. Brink*, M.M. Dewey, and D. Colflesh*, Anatomical Sciences, SUNY at Stony Brook, Stony Brook, N. Y. 11794.

The fluorescent dyes, fluorescein, aminofluorescein, dichlorofluorescein, dibromofluorescein, diiodofluorescein and tetrabromofluorescein, have been iontophoresed into the median giant axon of the earthworm nerve cord. The giant axon contains septa which have nexuses. The iontophoretic period lasted 30 minutes. Cytoplasmic binding of the dyes was determined by a Bio-rad dialysis apparatus. Most dyes showed no significant binding. The dyes reveal the cellular diameter and length allowing calculation of the cell volume. Dyes were iontophoresed into a microcurvette (30 ul) containing 0.2 M KCl. A dual beam spectrophotometer was used to determine the concentration of dye as compared to standard curves for various dye concentrations. Because the iontophoretic periods for dye injection into the microcurvette and cell were the same, the concentration of dye in the cell can be determined. Microdensitometric tracings were made from photographs taken just after dye injection termination and at varying time intervals (15 and 30 mins). Calculation of the shift in area under the curve from one side of a septum to the other with time and an estimate of dye concentration allows calculation of the permeability of the septum to the dyes (Permeability = flux/dye conc.). The dyes permeate the septa in the range of $10^{-6}$ thru $10^{-8}$ cm/sec. The presence of lanthanum (20 mM) in the extracellular media causes fluorescein diffusion through the septum to stop suggesting that the lanthanum uncouples adjacent cells of the median giant axon. The lanthanum blocks action potential propagation and causes marked membrane depolarization (see Kensler, R., P. Brink and M.M. Dewey this Meeting).

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**TH-POS-H4** MEASUREMENT OF RED CELL VOLUME AND AREA USING MICROPIPETTES. A.W.L. Jay, Division of Medical Biophysics, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Direct measurements of individual erythrocyte geometry (1,2) requires assumption of cylindrical symmetry. This makes the technique not applicable to conditions in which red cells have irregular or crenated shapes, such as in hyperosmolality or in the presence of chemical agents or drugs. A technique has been developed to measure the area and volume of individual red cells when they are held at the end of cylindrical glass micropipettes (diameter 1 - 2 μm) with small negative pressures. The membrane is smooth and the cell assumes a florence-flask shape with cylindrical symmetry. Previous experiments (3) have demonstrated that membranes from normal erythrocytes will not stretch as the cell is drawn into these pipettes. Study of a normal blood sample for which the mean cell area ia measured using the photographic technique (1,2) provides an accurate determination of pipette diameter (± 0.02 μm). Subsequent analysis of test samples using the same micropipette gives distributions of area and volume. This technique is currently used to study osmotic responses of red cells and to estimate the minimum (non-osmotic) cell volume in individual erythrocytes. This is correlated with the initial cell volume, providing distributions of minimum cell volumes and of osmotic water volumes in individual erythrocytes.

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**TH-POS-H5** TIME-DEPENDENT EFFECTS OF CHLORPROMAZINE ON RED CELL MEMBRANES. S.H. Roth* and A.W.L. Jay, Division of Pharmacology and Therapeutics, Division of Medical Biophysics, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Chlorpromazine (CPZ) is a highly lipid-soluble drug that can protect red blood cells from osmotic hemolysis by expansion of the cell membrane. These earlier studies using Coulter Counter sizing techniques on resealed RBC ghosts (1) reported increases in membrane area of 2 to 6 percent. Membrane expansion in single intact red cells can be measured directly using microphotographic techniques (2,3). Recent studies using these techniques indicate that CPZ does not induce expansion in all cells within a blood sample. In addition, most cells assume the cup-shape on initial contact with CPZ, with the majority of these cells reverting to the normal biconcave shape within 30 minutes. Crenation occurs upon washing with normal Ringer solution. This crenation is reversed by re-exposure to CPZ. These results stress the importance of the time-dependence of drug-membrane interactions.

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Supported by the Alberta Heart Foundation
Dr. Jay is a Research Scholar of the Canadian Heart Foundation.

**TH-POS-H6** ELECTROPHORETIC DETECTION OF REVERSIBLE CHLORPROMAZINE·HCl BINDING AT THE HUMAN ERYTHROCYTE SURFACE. T.S. Tenforde, J.P. Yee,* and H.C. Mel, Lawrence Berkeley Laboratory and Group in Biophysics and Medical Physics, University of California, Berkeley, California 94720.

Exposure of erythrocytes (RBC) to the anesthetic chlorpromazine·HCl (CPZ) is known to induce a reversible shape change from a biconcave disc (discocyte) to a cup-shaped form (stomatocyte). Using microelectrophoresis, we have detected the binding of CPZ at the human RBC surface through its effect on cellular electrophoretic mobility, and have investigated the dependence of this effect on cell form and deformability. Incubation of RBC ( ~5 x $10^6$/ml) in 23 μM CPZ resulted in a reduction of negative electrophoretic mobility from the control value of - 1.11 + 0.01 μm/sec/V/cm to - 1.00 ± 0.02 μm/sec/V/cm (pH 7.4, ionic strength 0.155). This mobility change was completely reversed when CPZ was removed from the cell suspension by centrifugal washing. Increasing the CPZ concentration to 70 μM did not affect the mobility change. Fixation of the RBC with glutaraldehyde was found not to affect either the normal electrophoretic mobility of discocytes or the reduced electrophoretic mobility of CPZ-induced stomatocytes. When these stomatocytes were first fixed with glutaraldehyde, then subsequently washed free of CPZ, they retained the stomatocyte form while regaining a normal control electrophoretic mobility. The mobility change is therefore independent of the drug-induced shape change, but instead reflects a contribution to cellular surface charge density from the membrane-bound CPZ molecules. From the charge reduction, it is estimated that ≲ 1% of the human RBC surface area is occupied by CPZ molecules, under conditions where the electrophoretically detectable drug binding sites are saturated.

TH-POS-H7 DEPENDENCE OF NET CHLORIDE PERMEABILITY OF HUMAN ERYTHROCYTES ON MEMBRANE POTENTIAL. P.A. Knauf and P.J. Marchant‡ Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada, M5G 1X8.

When fresh human erythrocytes are treated with low concentrations of valinomycin in a medium containing 1 mM $K^+$, the net KCl efflux is proportional to the valinomycin concentration. At high valinomycin concentrations ($\gtrsim 1$ μM), however, the net KCl efflux becomes insensitive to further increases in valinomycin, but is almost linearly proportional to the net Cl permeability, $P_{Cl}$ (Knauf et al., J. Gen. Physiol., in press). If $K_o$ is increased, the net KCl efflux reaches a plateau at progressively lower valinomycin concentrations, as expected from the Goldman-Hodgkin-Katz equation. Instead of remaining constant, however, the apparent chloride permeability decreases with increasing $K_o$. By using cells whose internal $K^+$ had been partially replaced with $Na^+$ by treatment with PCMBS in a low $K^+$ medium, it was found that if the membrane potential, $E_m$, was held constant, $P_{Cl}$ did not change significantly when $K_o$ was increased from 40 mM to 140 mM. On the other hand, at constant $K_o$, a 26 mV change in $E_m$ caused a significant ($p < 0.001$) variation in $P_{Cl}$, demonstrating that $P_{Cl}$ depends on $E_m$, rather than on either $K_i$ or $K_o$. The $P_{Cl}$ value obtained at membrane potentials from 0 to +7 mV is 0.011 $min^{-1} \pm 0.001$ S.E.M. ($8.4 \times 10^{-9}$ cm/sec), significantly different at the $p < 0.001$ level from the value of 0.032 $min^{-1} \pm 0.002$ S.E.M. ($2.4 \times 10^{-8}$ cm/sec) at -96 to -106 mV, conditions under which $P_{Cl}$ is usually measured. When $E_m$ is increased from 0 to more positive values, $P_{Cl}$ also increases. In addition to their significance for models of net anion flow, these data also suggest that calculations of the expected changes in membrane potential when cation permeability is increased may be erroneously low, if based on $P_{Cl}$ values determined at -100 mV. (Supported by Medical Research Council (Canada) Grant MA 5149.)

TH-POS-H8 ADHESION OF PHOSPHOLIPID VESICLES TO CHINESE HAMSTER FIBROBLASTS: ROLE OF CELL SURFACE LIPID AND PROTEIN CONSTITUENTS. Richard E. Pagano and Masatoshi Takeichi*. Carnegie Institution of Washington, Dept. of Embryology, 115 W. University Pkwy., Baltimore, Md.21210

The adhesion of unilamellar lipid vesicles to Chinese hamster fibroblasts in suspension was used as a model system for studying membrane interactions. Vesicles comprised of dipalmitoyl lecithin (DPL) or dimyristoyl lecithin (DML) adsorbed to the surfaces of EDTA-dissociated cells, and could not be removed by repeated washings of the cells, but could be released into the medium by treatment with trypsin. In cells treated with $^3$H-DML or $^3$H-DPL vesicles, EM autoradiographic studies showed that most of the radioactive lipids were confined to the cell periphery. Transmission and scanning electron microscopy confirmed the presence of adherent vesicles and membrane fragments at the cell surface.

Studies of the temperature dependence of vesicle uptake showed that DML or DPL vesicle adhesion to EDTA-dissociated cells was 2-5X greater at 2° than at 37°C. In contrast, cells trypsinized prior to incubation with vesicles, showed no temperature dependence of vesicle uptake.

Adhesion of DML or DPL vesicles to EDTA-dissociated cells prevented the lactoperoxidase catalyzed iodination of two cell surface proteins (MW≈60,000) without affecting the labeling of other membrane proteins. Incubation of cells with $^3$H-lipid vesicles followed by polyacrylamide gel electorphoresis of the extracted cellular proteins and autoradiography showed that the $^3$H-lipid migrated preferentially with these 60,000 MW proteins.

These results suggest two pathways for adhesion of lipid membranes to the cell surface--a temperature sensitive one involving specific proteins, and a temperature independent one. These findings are discussed in terms of current models for cell-cell interactions.

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TH-POS-H9 CHARACTERIZATION OF THE CHEMILUMINESCENCE ASSOCIATED WITH PHAGOCYTOSIS IN RABBIT ALVEOLAR MACROPHAGES. P.R. Miles, P. Lee, L. Bleigh*, and T. Sweeney*, Dept. of Physiol. & Biophys., West Virginia University, Med. Ctr. and A.L.O.S.H., N.I.O.S.H., C.D.C., Morgantown, West Virginia 26506.

Chemiluminescence which is associated with the phagocytosis of zymosan particles can be elicited from rabbit alveolar macrophages. Chemiluminescence was measured as the counts per minute obtained in the tritium channel of a liquid scintillation counter operated in the out-of-coincidence mode. When alveolar macrophages are exposed to zymosan, chemiluminescence increases gradually to a peak response at 15 to 25 minutes and then slowly decreases to background level over the next 2 to 3 hours. Two possible opsonins, plasma and lavage fluid (i.e., fluid obtained during lung lavage) have no effect on the response. Chemiluminescence increases as the zymosan concentration increases until the particle to cell ratio reaches approximately 1:1 where the response saturates. The metabolic inhibitors, DNP, rotenone, $CN^-$, $F^-$, and IAA, very rapidly inhibit the chemiluminescent response long before they have any effect on cell viability. Adenosine (5mM) produces a 70% inhibition and ATP (0.3mM) completely abolishes chemiluminescence, but ADP and dibutyrl cyclic AMP have no effect. The response is also affected by the $Ca^{++}$ concentration, i.e., at 0.5 to 1.0mM $Ca^{++}$ chemiluminescence is maximal, but at 2.5mM $Ca^{++}$ the response is virtually abolished. In addition, the sulfhydryl-reactive compound, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which is too large to penetrate the membrane, causes a 70% inhibition of the chemiluminescent response. This could mean that a receptor which contains sulfhydryl groups is important for the initiation of the response. These data indicate that chemiluminescence in these cells is probably due to a metabolic response which is important in the phagocytic process.

**TH-POS-H10** THE EXCHANGE OF CYTOCHROME $b_5$ BETWEEN PHOSPHOLIPID VESICLES. M.A. Roseman*, P.W. Holloway*, M.A. Calabro* (Intr. by Rafael Rubio) Department of Biochemistry, University of Virginia, Charlottesville, Va. 22901.

Cytochrome $b_5$, an intrinsic membrane protein isolated from liver microsomes, binds readily to preformed phosphatidylcholine (PC) vesicles. Although the binding constant must be very large, we have discovered that $b_5$ rapidly exchanges between egg PC vesicles. The most direct evidence follows: 1) When preformed $b_5$-vesicle complexes are incubated with fresh vesicles, a new species (or population) arises which sediments in the analytical ultracentrifuge as a single boundary with a rate intermediate between those of the original components; 2) When the same kind of mixture is subjected to free boundary electrophoresis, no slow-moving component corresponding to pure vesicles is seen; 3) When $b_5$-vesicle complexes are subjected to sucrose density gradient centrifugation at $20^o$ (with or without the addition of fresh vesicles) the complex segregates into free vesicles, which may account for about half the lipid, and a continuous series of particles having increasing ratios of protein to lipid. This cannot represent a static distribution of the original population since a single boundary is always seen by sedimentation velocity. Rather, this is the kind of result we expect if $b_5$ exchanges between vesicles. The results described in experiments (1) and (2) cannot be attributed to fusion or aggregation of vesicle species, as indicated by one series of experiments employing light scattering techniques, and another in which the interaction of $K_3Fe(CN)_6$- and ascorbate-loaded vesicles was studied in the presence of $b_5$. Furthermore, the values of the sedimentation coefficients obtained in Experiment (1) strongly support this conclusion. These results have important implications for studies of the PC-$b_5$ system as well as the enzymatic reactions in which $b_5$ is involved in vivo. This research was supported by USPHS Grant GM 14628 and American Cancer Society Grant BC 71 E.

**TH-POS-H11** CHEMILUMINESCENCE ASSOCIATED WITH PHAGOCYTOSIS IN DOG ALVEOLAR MACROPHAGES. Ping Lee, P.R. Miles, G. Tsekouras*, and M. Smith*, Dept. of Physiol. & Biophys., West Virginia University Med. Ctr. and A.L.O.S.H., N.I.O.S.H., C.D.C., Morgantown, West Virginia 26506.

Chemiluminescence which is associated with phagocytosis has been studied in dog alveolar macrophages. The chemiluminescence is produced by incubation of $5x10^7$ alveolar macrophages with $4x10^8$ zymosan particles in Hank's Balanced Salt Solution at 37°C and measured as counts per minute in the tritium channel of a liquid scintillation counter with the coincidence circuit off. After addition of the particles to the medium, chemiluminescence increases gradually and reaches a peak in 5 to 15 minutes and then decays to background level in approximately 1 hour. Dog plasma is required in the medium to promote the chemiluminescent response, but the addition of lavage fluid (i.e., fluid obtained during the lung lavage), bovine serum albumin, or egg albumin has no effect. Chemiluminescence is inhibited by DNP (0.1mM), an uncoupler of oxidative phosphorylation, and adenosine ($5x10^{-4}$M) but is not affected by ouabain ($5x10^{-4}$M) or dibutyrl cyclic AMP ($4x10^{-5}$M). Isoproterenol and epinephrine, which are used as bronchodilators, inhibit chemiluminescence, especially during the initial phase of the response. However, after this initial inhibition isoproterenol causes an activation of light emission. This enhancement of chemiluminescence by isoproterenol is thought to be due to oxidation of the drug by hydrogen peroxide which is released from the cells. These data indicate that chemiluminescence is associated with phagocytosis in alveolar macrophages and that the light emission is probably due to a metabolic response of the cells which can be inhibited by epinephrine and isoproterenol.

**TH-POS-H12** ELECTROPHORETIC LIGHT SCATTERING SPECTRA OF CHROMAFFIN GRANULES AND A PLASMA MEMBRANE FRACTION FROM BOVINE ADRENAL MEDULLA. D. P. Siegel and B. R. Ware, Department of Chemistry, Harvard University, Cambridge, MA 02138, and D. J. Green and E. W. Westhead, Department of Biochemistry, University of Massachusetts, Amherst, MA 01003

Chromaffin granules are membrane-bound vesicles within the adrenal medulla which contain protein and the catecholamines epinephrine and norepinephrine. These catecholamines are released into the blood stream by exocytosis following fusion with the cell membrane. The mechanism by which the granules approach and fuse with the plasma membrane is not known, although a calcium requirement has been established. Electrophoretic light scattering is a technique in which the electrophoretic velocities of a suspension of particles are obtained by analysis of the Doppler-shifted frequency spectrum of the scattered light. The electrophoretic mobility, which is a function of the surface charge on the particle, can be calculated from the Doppler shift magnitude, the electric field strength, and apparatus constants. The shape and width of the peak in the frequency spectrum due to a particular species reflects both the surface charge heterogeneity of the species and its diffusion coefficient. The apparatus permits the rapid and objective determination of the electrophoretic mobilities of a heterogeneous mixture of microscopic or submicroscopic particles. We have determined the electrophoretic distributions of suspensions of bovine chromaffin granules at varying concentrations of ATP, $Ca^{++}$, and $Mg^{++}$. We have also determined the electrophoretic distributions of vesicles prepared from the plasma membrane of chromaffin cells of the bovine adrenal medulla under the same conditions. Finally we have analyzed mixtures of chromaffin granules and plasma membrane vesicles to study their association.

TH-POS-H13 THE EFFECT OF TRITON X-305 ON THE DIELECTRIC CONSTANT OF HYDROPHOBIC COMPARTMENTS IN LIVER PLASMA MEMBRANES. V. Glushko, C. Karp and M. Sonenberg, Sloan-Kettering Institute for Cancer Research, New York, N.Y., 10021.

The activities of membrane-bound enzymes, such as adenylate cyclase and ATPase, are known to depend on interaction with lipid components, which can be disrupted by nonionic polymeric detergents without producing irreversible denaturation. As a bulk property, the dielectric of the environment can affect hydrophobic interactions. In order to determine the significance of this parameter to membrane organization and function, the vibrational fine structure of pyrene monomer fluorescence was employed to evaluate changes in the dielectric constant of the liver plasma membrane hydrophobic compartment as a function of detergent perturbation. Pyrene was incorporated into liver plasma membranes with a partition coefficient in excess of two orders of magnitude; the distribution of fluorescence fine structure reflected a hydrophobic environment with a dielectric constant of 12-14. Treatment with the nonionic polymeric detergent, Triton X-305, produced a relative increase in the dielectric constant of the pyrene compartment to 27-30. This occurred in a sigmoidal fashion as a function of X-305 concentration between 0.01 mM (0.002%) and 0.28 mM. Quenching by 0.1 M KI maintained the sigmoidal pattern but shifted it to higher X-305 concentrations, indicating the existence of multiple pyrene compartments of differing accessibility to the aqueous phase. Even at 5 mM X-305 and 1 M KI, 10% of the pyrene remained in a hydrophobic, aqueous inaccessible environment. The maximum inactivation (50-58%) of $Na^+$, $K^+$ dependent ATPase was produced over the X-305 range (0.04-0.15 mM) found to affect the aqueous accessible pyrene compartment, suggesting that hydrophobic regions near the membrane surface may be involved in ATPase activity. (Supported in part by NIH grants CA-08748, CA-16889 and AM-18759).

TH-POS-H14 PROTON PULSED NMR STUDY OF THE CELL CONSTITUENTS OF APHANOTHECE HALOPHYTICA, A BLUE-GREEN ALGA. B. S. Wong*, D. M. Miller, and J. H. Yopp*, Departments of Physiology and Botany, Southern Illinois University, Carbondale, Illinois 62901

Pulsed NMR studies on the cell constituents (Plasmalemma, Cell Wall, and Thylakoids) isolated from A. halophytica, a hypersaline photoautotroph grown at high temperature, have revealed non-exponential relaxation for both spin-lattice relaxation time $T_1$ and spin-spin relaxation time $T_2$ over the temperature range 50°C to -50°C. Relaxation times measured at 27°C for constituents and whole algae are as follows.

| RELAXATION TIMES | WHOLE ALGAE | THYLAKOIDS | THYLAKOID SUPERNATANT | CELL WALLS | PLASMA MEMBRANES | MEMBRANE SUPERNATANT |
|---|---|---|---|---|---|---|
| $T_{1a}$ | 48.8 ms | 45.3 ms | 1441 ms | 39.6 ms | 32.0 ms | 598 ms |
| $T_{1b}$ | 11.6 ms | 14.1 ms | | 11.2 ms | 12.2 ms | |
| $T_{2a}$ | 10.1 ms | 12.7 ms | 315 ms | 1.5 ms | 3.9 ms | 194 ms |
| $T_{2b}$ | 2.9 ms | 3.6 ms | | 0.4 ms | 1.4 ms | |

If free-induction-decay signal height extrapolated to zero time is monitored as a function of temperature, both cell wall and thylakoid fractions suffered a distinct signal drop at -6°C, indicating the initial freezing out of the more loosely bound water; whereas for the plasmalemma the drop occurred at +8°C. The latter is probably due to a phase transition phenomenon involving the bulk of the lipids in the plasmalemma. From the data, it can be seen that the constituent whose relaxation times most closely resembled those of the whole algae is of the thylakoids. The quantity of total water strongly bound by the thylakoids (18%) agrees with an earlier finding that 14% of the cell water was strongly immobilized. As with the previous study, we concluded that the immobilization is due to the highly proteinaceous inner membranes. (Supported by NASA Grant NGR 14-008-026)

TH-POS-H15 DIELECTRIC DISPERSION OF CULTURED LYMPHOMA CELLS (L5178Y) IN SUSPENSION: DETERMINATION OF MEMBRANE AND INTRACELLULAR ELECTRICAL PROPERTIES BY MEANS OF A 'DOUBLE-SHELL' MODEL. A. Irimajiri, Y. Doida†, T. Hanai† and A. Inouye*, Dept. of Biophysical Sciences, SUNY, Buffalo, N.Y. 14226; Dept. of Physiology and Institute for Chemical Research, Kyoto University, Kyoto, Japan.

Frequency-dependent changes in the dielectric constant and conductivity of L5178Y cells suspended in culture media were measured over a range 0.01-100 MHz by a bridge method, and an attempt has been made to explain the observed dielectric behavior of cells by taking explicitly into consideration the involvement of large nuclei located in the cytoplasm. In fact, the gross dispersion curves were not simulated satisfactorily in terms of a 'single-shell' model such as employed thus far,[1,2] whereas a 'double-shell' model in which one additional, concentric shell phase was incorporated into the 'single-shell'model gave a much better fit between the observed and the predicted dispersion curves. Based on the latter model, we analyzed the raw data of dielectric measurements to yield a set of plausible electric parameters for the lymphoma cell: $C_M$= 1.0 $\mu F/cm^2$, $C_N$= 0.4 $\mu F/cm^2$, $\varepsilon_k \simeq 300$, $\varkappa_c/\varkappa_a \simeq 0.9$, and $\varkappa_k/\varkappa_c \simeq 0.7$. Here, $C_M$ and $C_N$ are the specific capacities of cytoplasmic and nuclear membranes; $\varepsilon$ and $\varkappa$ are dielectric constant and conductivity with subscripts a, c and k denoting respectively the extracellular, the cytoplasmic and the karyoplasmic phases.

1) Pauly, H., Schwan, H. P. (1959) Z. Naturforsch. 14b, 125.
2) Hanai, T., Koizumi, N., Irimajiri, A. (1975) Biophys. Struct. Mechanism 1, 285.

**TH-POS-H16** FREE RADICALS AND CARCINOGENESIS: NITROXYL FREE RADICAL FORMATION IN A NITROSOFLUORENE-UNSATURATED LIPID REACTION. Robert A. Floyd, Biomembrane Research Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104.

I report the first demonstration of a free radical formed when the carcinogen 2-nitrosofluorene is allowed to react with a lipid containing a carbon-carbon double bond. The reaction appears to involve a pseudo Dield-Alder mechanism of covalent attachment of the carcinogen to the lipid. The reaction occurs spontaneously, requires no catalyst and is not inhibited by a nitrogen atmosphere or metal chelators. This reaction appears to be of significance in the coupling of active carcinogen with the inheritance macromolecules. Supported in part by NIH research Grant 1 RO1 CA18591.

**TH-POS-H17** NITROXYL FREE RADICAL INTERMEDIATE IN RAT MAMMARY PEROXIDASE CATALYZED OXIDATION OF THE CARCINOGEN N-HYDROXY-2-ACETYLAMINOFLUORENE. D.L. Reigh*, M. Stuart* and R.A. Floyd (Intr. by J.J. Killion), Biomembrane Research Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104.

A peroxidase preparation from rat mammary gland, obtained by homogenization of whole gland tissue followed by ammonium sulfate precipitation, catalyzed the oxidation of N-Hydroxy-2-acetylaminofluorene (N-OH-AAF) in the presence of either one of the peroxides $H_2O_2$, cumene hydroperoxide or linoleic acid hydropeoxide. A nitroxyl free radical intermediate having a g value of 2.0063 and nitrogen splitting of 7.7-7.9 gauss was observed. This peroxidase preparation also catalyzed the $H_2O_2$ driven oxidation of p-phenylenediamine and guaiocol with substrate oxidation ratios unlike hemoglobin or methemoglobin. Isolated mammary parenchymal cells yielded a peroxidase enzyme apparently similar to that obtained from whole mammary gland. Several antioxidants including ascorbate, glutathione and propyl gallate inhibited the $H_2O_2$ driven oxidation of N-OH-AAF by the mammary gland peroxidase(s).

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**TH-POS-H18** SEPARATION AND SUBFRACTIONATION OF MICROSOMAL MEMBRANES BASED ON THEIR SURFACE PROPERTIES BY PARTITION IN AQUEOUS, TWO-POLYMER PHASE SYSTEMS. R. Ohlsson*, B. Jergil*, and H. Walter, Dept. of Biochemistry, University of Lund, Lund, Sweden.

Aqueous solutions of dextran and of polyethylene glycol when mixed give rise to immiscible, liquid two-phase systems useful for separating cells or membranes by partition. Depending on polymer conc. and salt comp. chosen one can, to some extent, select the surface properties (e.g. charge, hydrophobic) that determine the partition coefficient (Walter et al. BIOCHEMISTRY 15:2959, 1976). Smooth, light rough and heavy rough microsomal membranes obtained by sucrose gradient centrifugation were subjected to countercurrent distribution (CCD) in a phase system which reflects both charge and hydrophobic properties. The partition of the membranes was primarily between the interface and the bottom (dextran-rich) phase, and CCD was carried out by separating the material in top + interface from that in the bottom phase. Smooth microsomes were almost exclusively at the interface; heavy rough membranes in the bottom phase and light rough preps. were intermediate. CCD patterns show complete separability of smooth and heavy rough microsomes as well as the bulk of heavy rough from light rough. The distribution of light rough microsomes points up considerable heterogeneity (2-3 peaks) with the right peak usually overlapping that of smooth membranes. Results of preliminary expts. reveal that some specific enzyme activities change under the CCD curve of the smooth microsomes, indicating a membrane subfractionation. Application of the cross-partition technique (see: Ericson, BIOCHIM. BIOPHYS. ACTA 356:100, 1974) to the membranes gives the isoelectric point of smooth and light rough microsomes as approx. pH 4.2 while that of heavy rough preps. is about 0.5 pH unit lower.

**TH-POS-H19** A MODEL OF THE CHOLINERGICALLY-ACTIVATED CHANNEL OF FROG SKELETAL MUSCLE. H. R. Guy, Dept. of Biological Sciences, State University of New York at Albany, Albany, N.Y. 12222.

The model is based on previously reported measurements of the permeability of the post-synaptic channel to various organic cations and the possible blocking effects of certain larger organic cations. The channel wall is assumed to be formed by three parallel peptide chains in a right handed triple helix. Each chain has twelve residues per turn. An ideal peptide sequence for this structure is $(Pro,Ala)_n$; however, other residues with hydrophobic side chains could be substituted for proline or alanine. The Pro side chain faces outward from the exterior wall of the channel while the Ala side chain is positioned between the backbones of the adjacent peptide chains. The carbonyl oxygen of Pro forms a H bond with the amide nitrogen of Ala in an adjacent chain. The carbonyl oxygen of Ala is located on the interior wall of the channel and faces inward. The channel contains one water molecule for each of the Ala oxygens. Every hydrogen atom of the water forms a H bond with either an Ala oxygen or an oxygen of another water molecule. When all of the hydrogens of the water are bound to the Ala oxygens, permeant cations such as tris(hydroxymethyl)amino-methane, biguanide, and bis(2-hydroxyethyl)dimethylammonium can pass through the channel without displacing any of the water; however, larger impermeant cations such as bis(2-hydroxyethyl)ethylmethylammonium cannot. The channel should be blocked by organic cations such as 2,4,6 triaminopyrimidine which can bind to the Ala oxygens or SKF-525 iodomethylate which possess apolar moieties that can react with the hydrophobic regions between the Ala oxygens. Supported by N.I.H. National Research Service Award 5 F32 NS05059 NEUB and USPHS grant NS-07681.