M-Pos45 [^{3}H]NITRENDIPINE AND [^{3}H]D888 BINDING TO TRANSVERSE TUBULES IS DECREASED IN MALIGNANT HYPERTHERMIA. J.M. Ervasti[a], M.T. Claessens[b], J.R. Mickelson[b] and C.F. Louis[a,b], Depts. of Biochemistry[a] and Veterinary Biology[b], University of Minnesota, St. Paul, MN 55108.

Transverse tubule (TT) vesicles have been isolated from the skeletal muscle of normal and malignant hyperthermia susceptible (MHS) pigs. MHS and normal TT did not differ in a number of properties including cholesterol or phospholipid content, ($Na^+ + K^+$)-ATPase or Ca^{2+}-ATPase activities, or [^{3}H]ouabain or [^{3}H]saxitoxin binding (Ervasti et al., 1988; Biophys. J. 53, 336a). However, in the presence of micromolar calcium, the B_{max} for [^{3}H]nitrendipine binding to MHS TT at 22°C was significantly less than for normal TT (26.4 ± 5.4 versus 40.6 ± 3.7 pmol/mg protein respectively) while the binding affinities were not significantly different (K_D = 2.7 ± 0.6 versus 3.3 ± 0.5 nM for MHS and normal TT respectively). Furthermore, the B_{max} for (-)-[^{3}H]desmethoxyverapamil ([^{3}H]D888) binding to MHS TT was also less than that for normal TT (17.8 ± 7.0 versus 37.4 ± 5.9 pmol/mg protein for MHS and normal TT respectively). At calcium concentrations greater than 0.1 mM, there was a greater inhibition of [^{3}H]nitrendipine binding to normal than to MHS TT such that binding was now similar for both preparations. As with purified TT, [^{3}H]nitrendipine binding to homogenates of MHS muscle was also significantly less than to homogenates of normal muscle (109 ± 20, versus 211 ± 19 fmol/mg protein for MHS and normal TT respectively). We conclude that the decreased [^{3}H]nitrendipine and [^{3}H]D888 binding observed in MHS TT could be due either to an altered dihydropyridine receptor protein, or to a decreased content of this protein in MHS muscle. This TT abnormality could contribute to the abnormal sarcoplasmic Ca^{2+} regulation in MHS muscle. Supported by NIH grant GM-31382.

M-Pos46 D600 SHIFTS THE INACTIVATION CURVE AND DEPRESSES K-CONTRACTURES OF MALIGNANT HYPERTHERMIA SUSCEPTIBLE (MHS) AND NORMAL SKELETAL MUSCLES. Esther M. Gallant, Brian D. Roggow, Sue K. Donaldson* and Naomi S. Taus. Department of Veterinary Biology, Department of Physiology* and School of Nursing*, University of Minnesota, St. Paul, MN 55108.

We investigated whether MHS and normal pig skeletal muscles might differ in their sensitivity to the calcium antagonist D600. Bundles of MHS and normal muscle cells, intact from tendon to tendon, produce similar tetanic tensions. D600 (5-10 μM) caused a small but significant ($P<0.001$) depression of 150 mM K-contracture force in only MHS muscles at 37°C (0.37 ± 0.02 vs 0.29 ± 0.03 normalized to tetanic tension for control vs D600 pretreated K-contractures, respectively). Ten minute exposure to 5 μM D600 accompanied by subthreshold depolarization (15 mM K^+) significantly depressed subsequent K-contractures in both MHS (0.37 ± 0.02 vs 0.12 ± 0.01; control vs depolarized + D600) and normal muscles (0.15 ± 0.02 vs 0.01 ± 0.01; control vs depolarized + D600); D600-induced depression being significantly greater ($P<0.01$) for normal than for MHS muscles. This effect of D600 + subthreshold depolarization resulted in part from a leftward shift of the inactivation curve for both MHS and normal muscles; there was no change in activation threshold. Neither caffeine-contracture magnitude nor threshold was altered by D600 ± depolarization in either muscle type. Thus, D600 depresses tension responses elicited via TT depolarization but does not modify SR Ca^{2+} release elicited by caffeine. That MHS muscles were less sensitive to D600 in combination with depolarization than were normal muscles is consistent with the diminished binding of calcium antagonists by MHS T-tubule vesicles (Ervasti et al., FASEB J. 2:A394). (Supported by Muscular Dystrophy Association of America.)

M-Pos47 MODIFIED RYANODINE-BINDING PROPERTIES OF SARCOPLASMIC RETICULUM FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIGS. James R. Mickelson, Lynn A. Litterer and Charles F. Louis. (Intr. by R.E. Poppele) Dept. of Vet. Biology, University of Minnesota, St. Paul, MN 55108.

When compared to normal pig SR, SR from malignant hyperthermia susceptible (MHS) pigs exhibits an abnormal Ca^{2+} dependence of ryanodine binding at the low affinity Ca^{2+} site (greater Ca^{2+} concentrations are necessary to inhibit MHS SR ryanodine binding), as well as a higher apparent affinity for ryanodine at optimal Ca^{2+} (6 μM) [Mickelson et al., J. Biol. Chem. 263, 9310; 1988]. We have further characterized the differences between the MHS and normal SR ryanodine receptor by examining the effects of adenine nucleotides, caffeine, and ruthenium red on ryanodine binding. Scatchard plots performed at optimal Ca^{2+} demonstrated that in the presence of 10 mM ATP or 10 mM caffeine the B_{max} for ryanodine binding to MHS and normal SR did not differ (12-16 pmol/mg). However, the K_d of the MHS receptor for ryanodine in the presence of ATP or caffeine (6.0 and 28 nM respectively) was significantly lower than that of normal SR (8.5 and 65 nM respectively). When the Ca^{2+} dependence of ryanodine binding was determined in the presence of 5 mM AMPPNP, the $Ca^{2+}_{0.5}$ for stimulation of MHS and normal SR ryanodine binding was similar (0.27 vs. 0.41 μM), while the $Ca^{2+}_{0.5}$ for inhibition of MHS ryanodine binding (238 μM) was significantly greater than for normal (74 μM). Furthermore, MHS SR ryanodine binding was stimulated by lower caffeine concentrations than was normal SR ($C_{1/2}$ of 1.7 vs 3.4 mM), while MHS and normal SR ryanodine binding was inhibited by similar concentrations of ruthenium red ($C_{1/2}$ of 80 nM). These results are in agreement with the hypothesis that a defect in the MHS SR ryanodine receptor, resulting in an alteration in calcium channel gating properties, is responsible for the abnormal calcium releasing activity of MHS SR. Supported by NIH GM-31382.

M-Pos48 COMPARISON OF THE MAMMALIAN AND AMPHIBIAN SKELETAL MUSCLE RYANODINE RECEPTOR-Ca^{2+} RELEASE CHANNEL COMPLEXES. Qi-Yi Liu, F. Anthony Lai, Le Xu, Rodney V. Jones, Jeffrey K. LaDine and Gerhard Meissner. Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599-7260.

The Chaps-solubilized rabbit and frog muscle sarcoplasmic reticulum (SR) ryanodine receptors were isolated as large complexes of apparent sedimentation coefficient ~30S comprising high molecular weight protein subunits of M_r ~400,000. As previously reported for the rabbit muscle ryanodine receptor (Lai et al., Nature 331, 315, 1988), the 30S ryanodine receptor complex from frog muscle was similarly found to induce high-conductance channels permeable to Na^+, Ca^{2+} and Ba^{2+} ions when reconstituted into planar lipid bilayers (γ_{Na}=590 pS in 500 mM symmetrical Na^+ and γ_{Ba} = 95 pS in 50 mM Ba^{2+} trans). Subconductance states of 300 and 150 pS (in 0.5 M Na^+) were observed. The channels were activated by Ca^{2+} and ATP and inhibited by Mg^{2+} and ruthenium red. In accord with a recent report (Suarez-Isla et al., Biophys. J. 54, 737, 1988), inositol trisphosphate (5-20 μM) often activated the native and purified rabbit and frog ryanodine receptor channels recorded in planar bilayers. Comparable activation of Ca^{2+} release in vesicle $^{45}Ca^{2+}$-flux studies using native heavy SR membranes was, however, not apparent. Our results indicate that the frog and rabbit skeletal muscle ryanodine receptor-Ca^{2+} release channel complexes are structurally and functionally similar proteins. Supported by MDA Fellowship (FAL) and NIH grant AR18687.

M-Pos49 A DEFECT AFFECTING THE SARCOPLASMIC RETICULUM CALCIUM RELEASE CHANNEL DETECTED AT THE SINGLE CHANNEL LEVEL. M.D. Fill, J.R. Mickelson, J. Vilven, B.A. Jacobson, R. Coronado, and C.F. Louis. Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX and Dept. of Veterinary Biology, University of Minnesota, St. Paul, MN.

Malignant hyperthermia (MH) is a pharmacogenetic disorder resulting from an alteration in the regulation of skeletal muscle sarcoplasmic Ca^{2+} concentrations. The primary abnormality in MH-susceptible (MHS) muscle appears to be in the mechanism controlling calcium release from its intracellular storage sites in the sarcoplasmic reticulum (SR). In this report we demonstrate that although SR preparations isolated from MHS and normal pig muscle do not differ in the content of the calcium release channel/ryanodine receptor protein, or the Ca^{2+} concentration dependence for activation of calcium release, the rate constant for calcium release from the MHS SR vesicles at a given Ca^{2+} concentration is two to three times greater than from the normal SR. Single calcium release channels from native MHS and normal SR vesicles incorporated into planar lipid bilayers also demonstrate a similar Ca^{2+} dependence for channel activation, although a small increase in MHS single channel conductance was noted. The most significant difference between MHS and normal SR single channels, however, is that the Ca^{2+} concentration required to inactivate the MHS channels is approximately ten-fold higher than that required to inactivate the normal channels. Thus, the abnormal gating behavior of the MHS SR calcium release channel appears responsible for the increased calcium release activity of MHS SR, and likely underlies the etiology of this skeletal muscle disorder. Supported by NIH, MDA, and AHA.

M-Pos50 THE HALOTHANE-SENSITIVITY GENE PRODUCT IS CLOSELY ASSOCIATED WITH THE RYANODINE RECEPTOR PROTEIN. James R. Mickelson, Esther M. Gallant, Brian D. Roggow, Lynn A. Litterer, William E. Rempel and Charles F. Louis. University of Minnesota, St. Paul, MN 55108

Pigs heterozygous for the halothane-sensitivity gene did not exhibit the characteristic signs of a malignant hyperthermia (MH) episode in response to a halothane-succinylcholine challenge, although some metabolic responses were significantly altered when compared to homozygous normals [i.e., increased venous pCO_2, and arterial and venous K^+]. Upon exposure to halothane, intact muscle bundles from homozygous halothane-sensitive pigs (MH-susceptible) exhibited decreased tetanus tension, as well as increased tetanus half-relaxation time and contracture, and thus were clearly distinguished from muscle bundles of homozygous normal pigs. The contractile responses of muscles from heterozygous pigs resembled those of the homozygous normals except for the occurrence of small halothane-induced contractures (10 of 12 bundles). In the presence of 6 μM Ca^{2+}, the binding of [^{3}H]ryanodine to SR isolated from the homozygous halothane-sensitive pigs was of a higher affinity than to SR isolated from the homozygous normal pigs (K_d = 70-90 nM vs. 265 nM respectively). The SR from the pigs heterozygous for the halothane-sensitivity gene, however, demonstrated intermediate values for the affinity for [^{3}H]ryanodine (K_d = 192 nM). We conclude that although the heterozygous halothane-sensitive pigs are not MH-susceptible, they do represent a separate phenotype which is significantly different from the homozygous normals and the homozygous halothane-sensitives, both in vitro and in vivo. Furthermore, the alterations in SR [^{3}H]ryanodine binding in the pigs containing a single copy of the halothane-sensitivity gene demonstrates that the protein product of this gene is closely associated with, and perhaps identical to, the SR ryanodine receptor protein. Supported by NIH GM-31382 and the MDA.

M-Pos51 EFFECTS OF RYANODINE ON $[Ca^{2+}]_i$ IN MALIGNANT HYPERTHERMIA SUSCEPTIBLE SKELETAL MUSCLE FIBERS. [1]J.R. Lopez,[1]L. Parra,[2]P. D. Allen. (Introduced by S. Desai) [1]Centro de Biofisica y Bioquimica, IVIC, Caracas, Venezuela. [2]Department of Anesthesia, Brigham and Women's Hospital, Boston, MA.

We have studied the effects of ryanodine (R) on $[Ca^{2+}]_i$ in skeletal muscle bundles isolated from the peroneus longus obtained from normal (Yorkshire) and malignant hyperthermia susceptible (MHS) (Poland China) swine. $[Ca^{2+}]_i$ was measured with calcium selective microelectrodes. Resting $[Ca^{2+}]_i$ in the normal muscle fibers in the absence of (R) was 0.12 ± 0.01 μM (M ± SEM). In normals addition of R at concentration of 0.001, 0.01, 0.1μM did not produce any detectable change in the resting $[Ca^{2+}]_i$. Addition of R at concentration of 1 μM produced a slow significant increment of $[Ca^{2+}]_i$ to 0.42 ± 0.02 μM which reached a plateau in 15 min. R at concentrations of 10 and 100 μM also produced an increase of $[Ca^{2+}]_i$ (6 ± 2 μM and 10 ± 3 μM) and was also associated with a slow muscle contracture. In the MHS swine resting $[Ca^{2+}]_i$ was 0.29 ± 0.02 μM. Addition of R at any of the concentrations used above induced a dose dependent increase in $[Ca^{2+}]_i$ to 0.39 ± 0.03 μM, 0.86 ± 0.04 μM, 4 ± 2 μM, 8 ± 3 μM, 9 ± 4 μM and 16 ± 5 μM (0.001, 0.01, 0.1, 1, 10 and 100 μM) respectively. The slow muscle contracture was also observed at concentrations of R<0.1μM. Dantrolene (50 μM) inhibited the increment in $[Ca^{2+}]_i$ and muscle contracture observed in the presence of R in both group of muscle fibers. These results show that R induced an increment in myoplasmic $[Ca^{2+}]_i$ and muscle contracture in both groups of animals, but in the MHS group the required dose was much lower. These differences suggest the presence of an abnormal ryanodine receptor in the SR of MHS animals. (Supported by MDA, CONICIT S1-1277).

M-Pos52 DIRECT INHIBITION OF SMOOTH MUSCLE CONTRACTILE ELEMENTS BY CAFFEINE
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Mechanism of inhibitory effect of caffeine on smooth muscle contraction was examined. In vascular smooth muscle of rat aorta, caffeine inhibited contractions with only a small decrease in cytosolic Ca level as measured by fura-2-Ca fluorescence. Since the inhibitory effect of caffeine was much greater than that caused by forskolin, effect of caffeine may not be due solely to the cyclic AMP-dependent mechanism. Therefore, we further examined the effete of caffeine on contraction and phosphorylation of 20 kDa myosin light chain (MLC) in chicken gizzard smooth muscle. Caffeine (20 mM) inhibited the high K-induced contraction in intact muscle or contractions induced by cumulative addition of Ca (1 - 100 uM) in permeabilized muscle. Caffeine (5 - 40 mM) inhibited the phosphorylation of MLC induced by 10 uM Ca. Thiophosphorylation of MLC was also inhibited by 20 mM caffeine. The inhibition of MLC phosphorylation was enhanced by decreasing ATP concentration from 1 mM to 0.1 mM. Calmodulin activity as monitored by Ca-calmodulin-dependent erythrocyte membrane (Ca+Mg)-ATPase was not affected by 20 mM caffeine. These results indicate that caffeine inhibits smooth muscle contraction by a decrease in cytosolic Ca level and also by a direct inhibition of contractile elements. The direct effect of caffeine is not mediated by a cyclic AMP-dependent mechanism, and may be due to the inhibition of MLC kinase, but not to the inhibition of calmodulin nor the activation of phosphatase activity.

M-Pos53 ULTRASLOW CONTRACTILE INACTIVATION IN FROG MUSCLE FIBRES.
Carlo Caputo and Pura Bolaños. Centro de Biofísica y Bioquímica. IVIC, Apdo. 21827, Caracas 1020A, Venezuela.
Hodgkin and Horowicz (J. Physiol.153:386, 1960) reported that contractile repriming capacity was decreased after prolonged exposure to high K-solutions. We have studied in detail this phenomenon using short (1.5 mm) muscle fibers of the frog with a two microelectrode voltage clamp technique. Following depolarization from -100 mV to 0 mV, a fiber undergoes a contracture and then relaxes spontaneously; repriming for 1 second at -100 mV enables the fiber to develop a second response, whose amplitude depends on the duration of the first depolarization. With this procedure it is possible to show the buildup of a very slow inactivation process with a half time of about 35 seconds and completion in 80 to 120 seconds. In the presence of caffeine (0.5 mM), and lanthanum (50 μM), the time course of development of this slow inactivation process is prolonged. Following prolonged depolarizations (>100s), the inactivation curve, relating the extent of repriming with the membrane potential, during the repriming period of 1-2 seconds, appears to be shifted by about 30 mV toward more negative potentials, compared to the relationship obtained after short (<10s) depolarizations. Lanthanum, 50 μM, partially reverses this effect (by about 20 mV). This effect of lanthanum is probably not due to fixed charges interaction, since at this low concentration the tension voltage relationship is not affected. Supported by MDA.

M-Pos54 EFFECT OF SULFHYDRYL REAGENTS ON CHARGE MOVEMENT AND CONTRACTILE RESPONSES IN FROG MUSCLE FIBERS. Adom González, Pura Bolaños and Carlo Caputo. Centro de Biofísica y Bioquímica, IVIC, Apdo. 21827, Caracas 1020A, Venezuela.

We have studied the effect of the sulfhydryl reagents p-chloromercuribenzoic acid (PCMB), p-hidroximercuribenzoate (PHMB) and p-hidroximercuriphenylsulfonic acid (PHMPS) on intramembrane charge movement in frog skeletal muscle using the cut fiber preparation with the triple vaseline gap technique, and on contractile tension using the short fiber preparation with a two microelectrode voltage clamp. For charge movement measurements the internal solution contained in mM:120 Cs-Aspartate and 10 EGTA while the external medium contained in mM:70 $(TEA)_2SO_4$, 5 Rb_2SO_4, 8 $CaSO_4$, 5 $MgSO_4$ and TTX. With PHMPS (1 mM), charge movement (charge 1 and charge 2) was appreciably reduced or completely abolished after 20-30 minutes exposure. These effects were prevented or partially reversed by DL-Dithiothreitol (DTT, 2 mM). During the measurements of charge 1 in the presence of PHMPS, a large inward current was usually observed, which could not be blocked either by Nifedipine (30 μM) or Cd (2 mM), but disappeared when external N-Methyl-D-Glucamine (NMG) substituted for TEA. Similar results were obtained with PCMB and PHMB (0.3 - 0.5 mM). In contractile experiments, PCMB, PHMB and PHMPS also reduced or abolished tension in response to depolarizing pulses; these effects were also prevented or reversed by DTT. These results support the idea that intramembrane charge movement is the voltage sensor for Excitation-Contraction Coupling, and suggest that the molecular moiety responsible for charge movement contains sulfhydryl groups. Supported by MDA.

M-Pos55 PERCHLORATE INDUCED ACTIVATION OF SKINNED, SKELETAL MUSCLE FIBERS OF THE FROG. Michael Fill and Philip M. Best, Department of Physiology and Biophysics and UICOM, University of Illinois, Urbana, IL 61801.

Perchlorate ion (ClO_4) at millimolar concentrations shifts the voltage dependence of contractile activation of intact frog fibers to more negative potentials. This effect can be accounted for by a shift in the voltage dependency of transverse tubular (t.) membrane charge movement rather than any direct effect on the sarcoplasmic reticulum (s.r.). We have investigated the ability of ClO_4 to affect contraction of skinned fibers activated by ionic substitution or caffeine. Skinned fibers (plasmalemma mechanically removed) were stimulated by ionic substitution (replacing K proprionate by choline chloride), caffeine, or ClO_4. Solutions contained 100 mM salt, 5 mM CP, 2 mM MgATP, 1 mM Mg, 50 μM EGTA (pCa=7.3) and MOPS (pH=7.0). The curve relating the magnitude of an ionic stimulus to developed tension was shifted to the left by ClO_4. A stimulus that was below threshold under control conditions gave 50% of maximal tension when 0.8 mM perchlorate was added to the bath. ClO_4 initiated contractions directly when added to the bathing solution, with 1.0 mM ClO_4 causing half the activation of a maximal stimulation by ionic substitution in the same fiber. The ability of caffeine to stimulate contraction was unaffected by ClO_4. These results support the notion that ionic substitution in skinned fiber activates calcium release by normal physiological mechanisms that include depolarization of the t-membrane. Supported by NIH AR32082 and the M.D.A.

M-Pos56 METABOLIC SUPPORT FOR A CHEMICAL MECHANISM OF E-C COUPLING IN BARNACLE MUSCLE FIBERS

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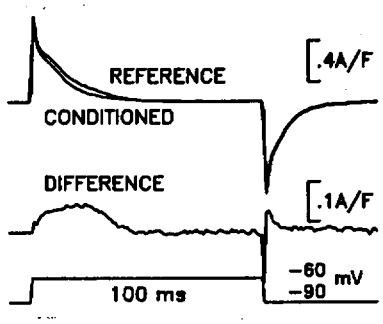
Giant barnacle muscle fibers from Balanus nubilus have a voltage dependent regenerative Ca conductance responsible for their excitable characteristics and for the entry of Ca^{2+} ions required to activate the contractile machinery. Optical experiments, performed in fibers voltage clamped and perfused internally with the Ca indicator azo1 suggest that, in addition to the entry of Ca^{2+} ions from the extracellular bath, there is a contribution from intracellular sources towards the change in intracellular Ca^{2+} concentration. This intracellular contribution is readily blocked by 1 mM neomycin, an inhibitor of the phosphoinositide metabolism. In support of the involvement of this cycle in the E-C coupling process in barnacle muscle, we found that in addition to PtdIns (PI) and PtdIns-4P (PIP), these muscles contain 39.6 and 10.3 nmol/g wet weight of PtdIns-(4,5)P_2 (PIP_2) and lysoPtdIns-P_2 (lyso-PIP_2) respectively. Their contents are sufficient to support the release of $InsP_3$ in response to electrical stimulation. Furthermore, we studied the incorporation of [^{3}H] myo-inositol, [gamma-^{32}P] ATP and [^{32}P]-H_3PO_4 into polyphosphoinositides in resting and electrically stimulated single giant muscle fibers and found that electrical stimulation causes a significant reduction in the specific activity of labelling of polyphosphoinositides, specially in PIP_2. Finally, although the isolated SR membrane preparation showed major specific [^{3}H]ryanodine binding in the nmolar range, internal perfusion with 10 μM ryanodine did not affect the Ca^{2+} transients recorded in single muscle fibers. Supported by grants from NIH, MDA and NSF. N.L. was a recipient of a Grass Fellowship and AHA-GLAA.

M-Pos57 **Qγ AND Ca RELEASE FLUX IN SKELETAL MUSCLE FIBERS.** L. Csernoch, I. Uribe, M. Rodríguez, G. Pizarro and E. Ríos (Introduced by Brenda Eisenberg). Rush University, Department of Physiology, Chicago, IL 60612.

A component of intramembrane charge movement was defined in cut skeletal fibers of the frog as the charge suppressed by any of three procedures: 1) a 50 ms conditioning pulse (Fig.); 2) exposure to low (20–40 μM) tetracaine; 3) a protocol of conditioning pulses shown by Schneider *et al.* (J. Physiol. 392) to cause Ca^{2+} depletion in the SR. The component so defined has a delayed time course that superimposes with the 'hump' visible in the records of total charge movement current. These procedures thus provide three approximately consistent definitions of Qγ, and show two new properties: 1) large disparity between the *on* (up to 6 nC/μF) and the *off* (about 1 nC/μF); 2) a non-monotonic dependence of Qγ on the voltage of the test pulse, with a maximum at –40 mV and decaying at higher voltage. These properties are simulated by a model in which Qβ (the fast component) and Qγ are produced by the same molecules (voltage sensors of EC coupling), and Qγ is driven by the depolarization caused by binding of intracellularly released Ca^{2+} to negative charges near the cytoplasmic face of the voltage sensors. According to this model Qγ is both *consequence* and *cause* of Ca release. Such a mechanism provides a positive feedback on Ca release that may contribute to the high steepness of release *vs.* voltage near threshold (Maylie *et al.*, J.G.P. 89). In conflict with this model, Qγ reaches peak current ≃ 3 ms earlier than Ca release flux. Supported by NIH and MDA.



M-Pos58 **PROTON GATING OF CALCIUM RELEASE CHANNELS IN VESICLES DERIVED FROM JUNCTIONAL SARCOPLASMIC RETICULUM.** Carmen Valdivia, Hector Valdivia, Janeen Vilven, and Roberto Coronado. Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

A calcium electrode assay was implemented to monitor Ca uptake via Ca pump and Ca release via ryanodine receptor release channels in "heavy" SR vesicles of rabbit skeletal muscle (fractionation procedure of Meissner, 1984). Typically 200 μg/ml SR vesicles in 1 mM ATP, 2 mM Mg, 0.3 M KCl, lowers free Ca from 3 μM to 0.3 μM in 120 s (initial rate was 20 nmoles/mg/sec). This uptake is stimulated by 2 mM oxalate, is insensitive to 50 μM oligomicyn and is releasable by 1 μM A23187 Ca ionophore. Peak of uptake is followed by a plateau and a slow release phase with a t1/2 of approximately 4 min at pH 7.5. No release of sequestered Ca occurred at pH 6.5. Single channel records in planar bilayers at similar free Ca showed that ryanodine receptor channels are mostly closed at pH 6.8 with open probability Po>0.01 but mostly open at pH 7.5, Po<0.3. Current carrier in low free Ca was 0.25 M CsCl. The pH-dependence of release in vesicles is explained on the basis of the pH-dependence of the release channel, mostly open at pH 7.5 but closed at pH 6.5. However, the delay in release following uptake suggests that Ca must first accumulate on the intravesicular space before a large fraction of channels in the vesicle population are available for opening. Thus, intraSR Ca may be an important variable controlling Ca release channels. We are currently comparing release waveforms in heavy SR and in intact triads. Supported by NIH, MDA, and AHA.

M-Pos59 **LIMITATIONS OF HISTOCHEMICAL IDENTIFICATION IN TRANSITIONAL SINGLE SKELETAL MUSCLE FIBERS.** C. E. Kasper & J. L. Thompson. School of Nursing, University of California, Los Angeles, Los Angeles, CA, 90024. Previous investigators have found that single peeled (sarcolemma removed) skeletal muscle fibers which are histochemically identified from their myofibrillar ATPase and oxidative staining patterns correlate with functional properties. Our aim was to determine if SF histochem techniques could be used to identify single peeled fibers from atrophied mammalian skeletal muscle. Adult female rats were subjected to 28 days of hindlimb suspension (HS) which resulted in decreases of 15% in mean body mass, 45% in mean SOL muscle mass (both significant @ $p \leq 0.05$). The maximum force-generating capacity of each fiber was examined using steady-state isometric force generation at saturating and subsaturating Ca^{2+} (pCa = 8,5.4,3.6; pH=7.0; 22±1°C), myosin isozyme composition (SDS-PAGE), and compared to fiber type from histochemical staining (myofibrillar ATPase @ pH 10.4 & 4.6, and NADH tetrazolium reductase) in a total of 80 single mechanically peeled single muscle fibers from atrophied and age-matched, non-suspended, control solew-twitch soleus (SOL). Each fiber was divided transversely into 5 segments. Four of the segments were used for histochemical staining and the remaining segment was used to measure isometric force-generation followed by analysis for myosin isozyme composition. The results demonstrate that the patterns of staining of peeled atrophied fiber segments are typical and distinguish type I from type II fibers. However, further subclassification of these fibers to types IIA and IIC were unreliable due to the marked increase in the relative amount of fast-type MHC's in these fibers. Supported by grants from NIH and NASA.

M-Pos60 EXTRACELLULAR MEASUREMENTS OF Na^+-DEPENDENT CALCIUM FLUX FROM GUINEA PIG ATRIAL TRABECULAE DURING CONTRACTURE. Chang Kook SUH and So Ra PARK, Department of Physiology, Yonsei University College of Medicine, Seoul, KOREA

The changes in extracellular calcium activities during contractures of guinea pig atrial trabecular muscles were measured with Ca^{2+}-selective electrodes. When the tissue was superfused with Na^+-free Tyrode solution, extracellular Ca^{2+} activities were decreased and contractures were induced with some delay. When the contracture was relaxed with Na^+-containing Tyrode solution, extracellular Ca^{2+} activities were increased transiently and recovered in a Na^+-dependent manner. As the concentration of Ca^{2+} in Na^+-free Tyrode solution was raised(from 1.0 to 5.0 mM), the recorded electrical signal(E^e_{Ca}) was decreased although the changes in extracellular Ca^{2+} activity was increased. The magnitude of extracellular Ca^{2+} activity decreased was proportional to the maximum magnitude of contracture induced by Na^+-free solution. Addition of caffeine(10 mM) to Na^+-free solution induced transient contracture following slow development of contracture and an increase in extracellular Ca^{2+} activity. Removal of caffeine from Na^+-free solution caused a slow relaxation of contracture and a decrease in extracellular Ca^{2+} activity. These results confirm that caffeine blocks Ca^{2+} uptake by the sarcoplasmic reticulum(SR) resulting in an increase in sarcoplasmic Ca^{2+} activity. Present experiments suggest that extracellular use of Ca^{2+}-selective electrodes provides continuous and quantitative monitoring of Na^+-dependent Ca^{2+} flux across the cardiac cell membrane.

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M-Pos61 BIOCHEMICAL CHARACTERIZATION OF FREE AND JUNCTIONAL TRANSVERSE TUBULES. E. Damiani, S. Salvatori, J. Barahnin*, G. Salviati, M. Lazdunski* and A. Margreth, NRC Unit for Muscle Biology and Physiopathology, Institute of General Pathology, University of Padova, Italy and * INSERM, Centre de Biochimie, University of Nice, France.

In skeletal muscle, T-tubule (TT) system consists of junctional and non-junctional tubules having different morphological features (Franzini-Armstrong, in Myology, Engel A.G. ed, p. 125, 1986). We have isolated junctional TT from triads according to Morgan & Kuypers (Arch. Biochem. Biophys. 253, 377, 1987), and "free TT" by sucrose density centrifugation from total microsomes (Sabbadini & Okamoto, Arch. Biochem. Biophys. 223, 107, 1983; Saito et al., J. Cell. Biol. 99, 875, 1984). We found that free TT were biochemically homogeneous, irrespective of the isolation procedure used, and contained a Mg-ATPase activity 3 to 10-fold higher than junctional TT. This result correlates with the electrophoretic protein pattern, showing a higher content of 100 kDa protein, previously tentatively identified as the Mg-ATPase, in free vs junctional TT. On the contrary, junctional TT showed a high density of PN-200-110 binding sites (50 pmoles/mg pr.), as compared to free TT (5-15 pmoles/mg pr). The distribution in junctional and free TT of cholesterol, beta-adrenergic receptor, Na-K ATPase (as determined by ouabain-binding) and of saxitoxin-sensitive Na-channel showed minor differences. Purified rabbit sarcolemma membranes significantly differed from both junctional and free TT. These results indicate that junctional and non junctional TT represents distinct areas of biochemical specialization of the T-system, and that Mg-ATPase activity and PM-200-100 binding are useful markers for discriminating the two membrane preparations.

M-Pos62 ENANTIOMERS OF A CHIRAL CLOFIBRIC ACID ANALOG DO NOT AFFECT CHLORIDE CHANNELS IN FROG SKELETAL MUSCLE. R. Wagner, S.H. Bryant, D. Conte-Camerino*, V. Tortorella*, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 and *Dipartimento Farmacobiologico, Facoltà di Farmacia, Università di Bari, 70126-Bari, Italy.

Enantiomeric pairs of a methyl derivative of 2-(p-chlorophenoxy)isobuteric acid (clofibric acid, or CPIB) have been shown to produce opposite actions on chloride conductance of rat skeletal muscle fibers (Conte-Camerino et al., *Pflugers Archiv* [1988], in press). At concentrations below 10 μM the S(-) enantiomer blocks while the R(+) enantiomer increases resting chloride conductance. The R(+) enantiomer effect in mammalian muscle occurs at concentrations of 1μM, which is much lower than effective concentrations of known skeletal muscle chloride channel blockers. The chloride conductance of frog skeletal muscle, on the other hand, has appeared to be relatively insensitive to the effects of chloride channel blockers such as anthracene-9-carboxylic acid (K. Woll et al.,*Pflugers Archiv* 410: 632-640, 1987.) These facts led us to use the enantiomers to test the hypothesis that the frog chloride channels have a low affinity receptor for this class of agents. Chloride conductance was measured in single semitendinosus muscle fibers from the bullfrog (*Rana catesbeiana*) *in vitro* at 20°C with standard two microelectrode methodology, as reported previously. Both R(+) and S(-) enantiomers, at concentrations as high as 1mM, produced no effect on chloride conductance. These results suggest that the bullfrog chloride channels are qualitatively distinct from the chloride channels of resting mammalian skeletal muscle in that the bullfrog channel lacks a specific aromatic carboxylic acid receptor. (Supported by NIH Grants NS-03178, TG HL 07382-12 and MDA Grants-in-aid.)

M-Pos63 AGENTS WHICH PROMOTE THE CONTRACTION AND RELAXATION OF THE ABRM OF Mytilus edulis DO NOT MODIFY pH_i. Simpson AWM, Ishii N* Ashley CC University Laboratory of Physiology, Parks Rd, Oxford OX1 3PT. UK. *Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, Japan.

We have recently demonstrated that in intact single cells $[Ca^{2+}]_i$ is elevated at the onset of a catch contraction and then declines to its resting level while tension remains high. Addition of serotonin (5-HT) relaxes tension without modifying $[Ca^{2+}]_i$. Studies on skinned fibres suggest that contraction may be dependant on pH as well as $[Ca^{2+}]_i$ with tension being promoted by acidification and relaxation by alkalisation. We have used the pH indicator BCECF to investigate whether carbachol (CCh), KCl or 5-HT modify pH_i. Smooth muscle cells isolated from the ABRM were incubated with 5μM BCECF/AM for 2h at 30°C. After loading, the cells were washed and resuspended in ASW containing 4mM Ca^{2+}. Suspensions were then excited at 490/450nm and emission monitored at 520nm. Neither 5mM CCh, 100mM KCl or 10μM 5-HT modified the resting pH_i which was calculated to be 7.13± 0.22 (mean±sem, n=6). Addition of 10mM NH_4Cl or 10mM $NaHCO_3$ elevated pH_i (+0.3-0.6 pH unit) and decreased pH_i (-0.4 pH unit) respectively indicating that the dye was monitoring pH_i. Under the microscope no change cell length was observed as a result of these additions. After acidification 5-HT still had no effect on pH_i and after alkalisation, again no change in pH_i was seen with either CCh or KCl. These results suggest that agents which promote contraction and relaxation do not alter pH_i and imposed changes in pH_i do not evoke contraction.

M-Pos64 SURFACE CHARGE EFFECTS ON T TUBULE CONDUCTION IN SKELETAL MUSCLE: A SIMULATION STUDY. John N. Howell and Anu Shankar, Department of Zoological and Biomedical Sciences and the College of Osteopathic Medicine, Ohio University, Athens, OH 45701

T-tubular conduction failure has been invoked to explain the decrease in tetanus tension and the changes in the early afterpotential (EAP) induced in frog skeletal muscle by elevated extracellular (Ca^{2+}) (Howell et al., J. Muscle Res. Cell Motil. 8:228, 1987). In order to see if these changes can be explained on the basis of the well-known surface charge effects of Ca^{2+}, we incorporated into the action potential simulation program of Adrian and Peachey (J. Physiol. 235:103, 1973) the Gouy-Chapman equation and the apparent binding constant for Ca^{2+} reported by Doerrscheidt-Kaefer (Pflueger's Arch. 360:171, 1976). Eighty millisecond simulations were run on the Cray at the Ohio Supercomputer Center. Although the timing and configuration of the notch and hump of the EAP are sensitive to a number of variables in the model, two persistent effects of high Ca^{2+} are to increase the amplitude of the EAP as in the laboratory measurements and to decrease penetration of the action potential into the t-system sufficiently to account for at least part of the reported decrease in tetanus tension. The model fails, however, to predict the measured changes in $T_{\frac{1}{2}}$ of the early afterpotential, suggesting that there may be other processes at work that are not included in the model.

(Supported by the American Osteopathic Association and the Ohio University College of Osteopathic Medicine.)

M-Pos65 MONITORING THE REGULATION OF SARCOPLASMIC RETICULUM TRANSPORT RATE INDIRECTLY BY TENSION INDEPENDENT HEAT FROM RABBIT PAPILLARY MUSCLES. EM Blanchard, NR Alpert. Dept of Physiol & Biophys, University of Vermont, Burlington, VT 05405.

Activation and relaxation of the myocardial cell requires the release and active sequestration of free calcium ions in the μM range. Calcium transport rate by the sarcoplasmic reticulum (SR) can be directly controlled by substrate concentration according to Michaelis-Menten behavior (velocity=Vmax x [Ca]/Km + [Ca]) or indirectly regulated by changes in the level of phosphorylation of phospholamban (possibly at ser-16 and/or thr-17) associated with the SR Ca-ATPase protein. We tested the hypothesis that SR transport rate in rabbit right ventricular papillary muscles is increased to a rate greater than predicted from increased substrate concentration alone when the muscles are incubated in a Krebs solution containing either 1 μM isoproterenol (cAMP dependent phosphorylation of phopholambam) or 11 mM calcium (calcium/calmodulin dependent phophorylation of phospholambam). We monitored SR transport rate indirectly by measuring the initial linear rate of tension independent heat (TIH) obtained with low thermal capacity, antimony-bismuth thermopiles (21° C, 0.2 Hz). TIH was isolated with 2,3-butanedione monoxime (5 mM) and mannitol (362 mM). We predicted that the increase in peak free Ca associated with the 36% increase in peak twitch tension caused by both interventions should increase initial TIH rate by 24%. Initial TIH rate was increased by 78+16% and 38+12% compared to controls for 1 μM isoproterenol and 11 mM calcium, respectively. These data suggest that isoproterenol increases the rate of uptake above that predicted from the Michaelis-Menten relationship by a cyclic AMP dependent mechanism while the increase in uptake rate following incubation in high calcium is explainable through the Michaelis-Menten mechanism. Supported by PHS #28001/06/P1.

M-Pos66

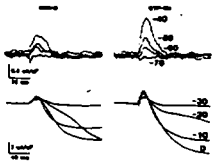
LONG RANGE ELECTRON TRANSFER IN PROTEINS AND POLYPEPTIDES. THE CONSTRUCTION OF MOLECULAR "WIRES"? Michael R. DeFelippis, M. Faraggi, and Michael H. Klapper; Biological Chemistry Division, Department of Chemistry, The Ohio State University, Columbus, OH 43210, and Department of Chemistry, Nuclear Research Centre-Negev, Beer-Sheva, 84190, Israel

Although Long Range Electron Transfer (LRET) in proteins is by now a well known phenomenon, it is still not well understood. In particular, we have little information on the polypeptide LRET distance dependence and equilibrium driving force (E_o) dependence, nor do we know the influence of the intervening medium on the LRET rate. Since LRET in proteins has an obvious importance in physiological electron transfer, we have undertaken a study of LRET between tryptophan (trp) and tyrosine (tyr) residues in a number of synthetic peptides.

The pulse radiolytically generated trp side chain indolyl radical can oxidize the tyr side chain to the phenoxy radical. Since both radicals absorb light in the visible region, we can monitor the 1-electron intramolecular transfer between them and determine 1st-order LRET rate constants. Together with this information we have also measured the redox potentials of these two radicals by both pulse radiolysis and electrochemical methods. Our results with peptides in which -(proline)$_n$- spacers separate the tyr and trp residues suggest that as the redox potential difference between electron donor and acceptor becomes smaller the LRET rate constant dependence on the chain length also becomes smaller; i.e., the peptide "conductance" appears to increase as $\Delta\varepsilon$ falls. This observation suggests that polypeptides might be able to act as "wires".

M-Pos67 **EFFECT OF GTP-g-S ON CHARGE MOVEMENT AND CALCIUM CURRENT FROM FROG AND RAT SKELETAL MUSCLE.** R. Gamboa-Aldeco*, J. García & E. Stefani. Dept. Physiology & Molecular Biophysics. Baylor College of Medicine. Houston TX, 77030.

G proteins directly couple membrane receptors to K and Ca channels in cardiac muscle (1,2) and modiffy the response of DHP receptor in neurones. Since it has been suggested a possible role of DHP receptors in excitation contraction coupling in skeletal muscle, we studied the possible participation of a G protein in this process. Voltage clamp experiments were made in cut single fibres from rat EDL and frog semitendinosus muscles using the vaseline gap technique. $[Ca]_o$ was 2 mM (rat) or 10 mM (frog). We found that intracellular concentrations from 10 to 100 μM of GTP-g-S, a nonhydrolyzable analog of GTP which activates G protein, caused an increase of the charge movement (40 % at -30 mV in rat; 37 % at 0 Mv in frog) and of the Ca current (I_{Ca}) (10 to 100 % peak tail current at -10 mV in rat; 150 % at 0 mV in frog). On the other hand, 2 mM external db-cAMP did not affect charge movement while it potentiated I_{Ca}. This indicates that charge movement in the skeletal muscle is regulated by G protein. GTP-g-S increase charge movement at potentials more negative than -30 mV where a fraction of the charge is probably related to the E-C coupling. Supported by NIH. * Universidad Juarez Autonoma de Tabasco Fellowship. 1. A. Yatani et al.(1987). Science 235: 207. 2.A. Yatani et al. (1987). Science 238: 1288.



M-Pos68 IRREVERSIBLE MODIFICATION OF EC COUPLING IN MAMMALIAN MYOCARDIUM AFTER ENDOCARDIAL DAMAGE. J.P. Bourreau, H.S. Banijamali and C.E. Challice. Physics Department, University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

It was recently reported that the removal or damage of the endocardial layer from mammalian myocardium significantly modifies both contraction and relaxation (Med. Sci. Res. 16: 577-578, 1988). Experiments were undertaken to provide further documentation on this effect. Cat papillary muscle from the right ventricle were used (diameter 0.6 → 1.2 mm), with great care being taken to avoid damage of the endocardial surface. Muscles were pinned horizontally to the bottom of an organ chamber by the septum end, with the tendinous end attached to the look of a mechano-electrical transducer (isometric recording). Muscles were stimulated at 0.2 Hz at 32 °C in a salt solution containing 1.0 mM Ca^{++}. The muscle length was adjusted until the maximum length-tension relationship was obtained. After 60 minutes stabilisation, transmembrane potentials and the associated contractions were recorded. After a 5-10 minute stable impalement, a 100 μl Triton x 100 (1% by volume) bolus was injected into the 3.5 ml chamber superfused at 20 ml/min. This resulted in a quick irreversible drop in peak force. The time-to-peak tension, and the time to half relaxation, were shorter following triton treatment, and in parallel, action potential duration was shortened and amplitude increased. This phenomenon showed a strong dependency upon extracellular Ca^{++} concentration and tended to disappear in high extracellular Ca^{++} concentration. A tentative hypothesis suggests either an endocardium-dependent regulation of the slow inward Ca^{++} current and/or an endocardium dependent control of a Ca^{++}-dependant K^+ permeability. (Supported by a grant from NSERC Canada).

M-Pos69 THE TnCDANZ FLUORESCENCE INTENSITY TRANSIENT AFTER FLASH PHOTOLYSIS OF "CAGED ATP". K. Guth[1], H. Rensland[2], R.S.Goody[2] and James D. Potter[3] [1]EMBL, 6900 Heidelberg, F.R.G., [2]Max-Planck -Institut fur medizinische Forschung, 6900 Heidelberg, F.R.G.and [3]Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

DANZ labelled TnC was incorporated into rabbit psoas fibers. We have previously shown (Guth and Potter (1987) J.Biol.Chem.) that the TnCDANZ fluorescence is increased when the Ca concentration is increased or when rigor crossbridges are formed. It was concluded that the TnCDANZ signal may reflect the regulatory state of the thin filament. The experiments reported here were performed in order to investigate the time course of the change of the regulatory state of the thin filament when the muscle is rapidly transferred from the rigor into the relaxed state by means of flash photolysis of caged ATP to ATP. Small bundles (3-4 fibers) were incubated in an ATP-and Ca^{2+}-free solution and then transferred into the rigor state. After the rigor force had completely developed, the fibers were incubated in the same solution containing additionally caged ATP (2mM). Shortly before the UV flash (xenon flash lamp), the fiber bundle was removed from the solution. After the flash, a typical mechanical relaxation transient was seen. If the rigor force was decreased shortly before the flash, by allowing the muscle to shorten, a transient force increase was observed before relaxation. The fluorescent intensity was measured simultaneously, and was seen to decay as a single exponential regardless of whether a transient force increase was seen. The fitted rate constants for the fluorescence decay and for the later part of the mechanical relaxation were identical. The addition of 10 mM inorganic phosphate to the solution approximately doubled the rates of both transients. This work was supported in part by the NIH and MDA and Deutsche Forschungsgemeinschaft.

M-Pos70 VOLTAGE DEPENDENT SARCOPLASMIC RETICULAR CALCIUM LOADING IN THE ABSENCE OF INWARD CALCIUM CURRENT IN FELINE VENTRICULAR MYOCYTES. Wm. H. duBell and S. R. Houser. Dept. of Physiol, Temple Univ. Sch. of Med, Phila, PA 19140

Calcium (Ca) which enters the cell during the cardiac action potential can be taken up by the sarcoplasmic reticulum (SR) for release in subsequent contractions. The present study examined whether SR Ca loading could occur by a voltage-dependent mechanism which is independent of the inward "L type" Ca current. Whole cell voltage clamp techniques were used to control membrane potential while intracellular Ca (Ca_i) was monitored with Indo-1 and Ca current was blocked with Verapamil. Resting myocytes held at -40mV were depolarized to test potentials between 0mV and +100mV for 5-10 sec. Depolarizing steps in the presence of Verapamil failed to evoke any detectable inward Ca current or a Ca transient. These steps did produce a slow rise in Ca_i which increased as the test potential was made more +. Prolonged depolarizations led to spontaneous Ca transients that most likely resulted from spontaneous Ca release from a Ca overloaded SR. The time from the beginning of the test pulse to the spontaneous Ca transient decreased as the membrane potential was made more +. Transient membrane currents associated with these spontaneous Ca transients were outward at potentials + to +40mV and inward at more - potentials. These results show that SR Ca loading can occur via a voltage dependent process that may involve the Na/Ca exchanger. (Supported by NIH HL33921 and HL33648 to SRH)

M-Pos71 ^{45}Ca UPTAKE DURING RECOVERY FROM A PRECEDING CONTRACTURE. B.A. Curtis, University of Illinois College of Medicine at Peoria, Peoria, IL 61656.

Curtis & Eisenberg (1985) described three components of Ca influx including a contractile related Ca influx occurring after contraction and suggested it was refilling a Ca store emptied to initiate the preceding contracture. To test if this Ca influx is related to the recovery necessary before a second contracture can be elicited, ^{45}Ca was added for varying times during the recovery period (90% complete in 70 sec at 12°C). After a 190 mM $KMeSO_3$ contracture, bundles of 3-5 twitch muscle fibers were rapidly rinsed with Ringer for 10-15 sec and ^{45}Ca Na added. After 30 or 60 sec the bundles were rinsed and moved over a window. Bundles exposed to ^{45}Ca for 30 sec (10-40 sec of recovery) took up 1.9 pM Ca/fiber. When exposed for 60 sec (10-70 sec of recovery) they took up 3.8 pM/fiber. Bundles exposed before and during contracture took up 2.9 pM Ca/fiber. Following exposure to ^{45}Ca during the recovery period ^{45}Ca left the bundles rapidly (t_c of 15 min) while following exposure during the contracture, loss was slow (t_c of 144 min). The different time constants of efflux suggest ^{45}Ca loss from two different compartments; a rapidly exchanging Ca store on the t membrane and a slowly exchanging Ca store in the SR.

Supported by American Heart Association, Illinois Division.

M-Pos72 LYOTROPIC ANIONS: EFFECTS ON Ca^{2+} CURRENTS, CHARGE MOVEMENT AND Ca^{2+} TRANSIENTS IN FROG SKELETAL MUSCLE. D. García-Díaz[+], M. Delay[++] and J.A. Sánchez[+++]. [+]Department of Physiology, Faculty of Medicine, UNAM, [++]Department of Medical Physiology, University of Calgary, Alberta, Canada and [+++]Department of Pharmacology, CINVESTAV-IPN, Mexico City.

Lyotropic anions facilitate activation of charge movement in skeletal muscle (García-Díaz & Sánchez, Biophys. J. 33, 334a, 1988) the present experiments examine further its action on charge movement, slow Ca^{2+} currents (I_{Ca}) and Ca^{2+} transients. Methods: The triple vaseline gap technique (Hille & Campbell, J. Gen. Physiol. 67, 265, 1976). Solutions (mM): For voltage clamp experiments: External: Mg^{2+} or Ca^{2+} = 10, TEA = 108, TTX = 0.1, T = 15 or 20°C. Internal: TEA_2EGTA = 20, Cs^- glutamate = 89, Na_2ATP = 2, MOPS = 4, Ca^{2+} transients were measured under current clamp conditions in Cl^- Ringer's. Internal solutions: EGTA = 1, K-aspartate = 121.5, $MgCl_2$ = 2, MOPS = 5, Na_2ATP = 2 and T = 22-24°C. Arzenazo III (1 mM) or Antipiralazo III (2 mM). The potentiometric dye NK2367 was superfused in the A pool. Results: Time to peak of I_{Ca-s} during large depolarization (ms) was 512 ± 80(14) and 330 ± 36 (14) after SCN^-. I-V curve shifted -21 ± 2.7 mV (14) after SCN^-. On-charge (mV) shifted from -39.9 ± 9.1(6) to -50 ± 2.0(6). Internal SCN^- produced similar results on I_{Ca} and charge movement. Tail currents were fitted with 2 exponentials (ms): τ_1 = 15.1 ± 2.8(11) and 67.0 ± 20.4(11) after SCN^-. Off-charge step to -10 mV) was slowed down ca 2.4 times. Peak amplitude of Ca^{2+} transients increased by 70-100% by SCN^- in both Arsenazo and Antipiralazo experiments. The rise time was basically unaffected. In contrast, a decrease of ca. 30% was observed in the decay rate. SCN^- did not produce marked alterations in T system electrical properties measured with NK2367.

Supported by CONACyT, Mexico and Heritage Foundation, Canada.

M-Pos73 THE CALCIUM DEPENDENCE OF CALCIUM UPTAKE BY SARCOPLASMIC RETICULUM IN VOLTAGE CLAMPED SKELETAL MUSCLE FIBERS. L. Kovacs, M.G. Klein, B.J. Simon and M.F. Schneider, Dept. of Biological Chemistry, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Myoplasmic [Ca^{2+}] was monitored simultaneously at two different sensitivities using the two indicators antipyrylazo III and fura-2 in voltage clamped cut frog skeletal muscle fibers (8-10 °C). The rate of release (R_{rel}) of calcium from the sarcoplasmic reticulum (SR) during depolarizing pulses was calculated from the [Ca^{2+}] transients. Conditioning pulses were used to deplete about 1/3 to 1/2 of the SR calcium content. Assuming R_{rel} to be proportional to SR content, fractional depletion was calculated as the suppression of release in a test pulse applied more than 1 s after the conditioning pulse (J. Physiol., 392:167, 87) to allow recovery from release inactivation (J. Physiol., 405:727, 88). From 2 to 16 s after the conditioning pulse [Ca^{2+}] declined from 83 ± 6 nM (mean ± se, n = 11) toward its resting level (39 ± 3 nM). The rate of net calcium uptake by the SR in the same fibers was determined as the rate of recovery from depletion using test pulses applied 2 to 16 s after the conditioning pulse. From 2-4, 4-8 and 8-16 s the uptake rates were 1.90 ± 0.21, 0.88 ± 0.13 and 0.49 ± 0.08 % of the initial SR calcium content/s at mean [Ca^{2+}]'s of 77 ± 5, 67 ± 4 and 57 ± 3 nM. From the extent of depletion during the conditioning pulses the mean initial SR calcium content was estimated to be about 1000 µM (referred to myoplasmic volume), giving uptake rates of 19, 9 and 5 µM/s. In the average data and in the data from each fiber the rate of calcium uptake by the SR increased more steeply with [Ca^{2+}] than a linear dependence and exhibited no evidence of saturation for [Ca^{2+}] up to 100 nM. (Supported by NIH 2PO1-HL 27867 and NSF DCB 8544787).

M-Pos74 FLUORESCENCE DECAY OF SARCOPLASMIC RETICULUM ATPase: LIGAND BINDING AND HYDRATION STUDIES. Sergio T. Ferreira and Sergio Verjovski-Almeida, Department of Biochemistry, Institute of Biomedical Sciences, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21910, Brazil

Intrinsic fluorescence decay of Ca^{2+}-ATPase was studied through phase-modulation fluorometry. Ca^{2+} binding to ATPase high affinity sites produced a 5% increase in the average lifetime. The decay was complex and was fitted by three exponentials. Data were fitted equally well by bimodal Lorentzian distributions of lifetimes. Ca^{2+} binding in the presence of Mg^{2+} shifts both components to longer lifetimes. ATPase fluorescence was studied as a function of hydration in reverse micelles of detergent AOT in hexane. The emission spectrum of ATPase at w_o=0.3 mol H_2O/mol AOT was blue shifted by 4 nm with respect to that in water. The average lifetimes and integrated intensities of emission decreased in parallel as hydration increased from w_o=0.3 to 5 suggesting that a dynamic quenching mechanism may be involved. The lifetime of FITC-labeled ATPase in reverse micelles decreased with increasing amounts of water in a biphasic manner. Low water (w_o=2.1 to 5.5) causes 50% of the maximal decrease in lifetime. The remaining decrease is only attained in a much higher hydration range (w_o=5.5 to 60) probably reflecting some difficulty in penetration of water into the active site. Supported by grants from CNPq and Finep, Brazil.

M-Pos75 LOCALIZATION OF MALEIMIDE-DIRECTED SPECTROSCOPIC PROBES IN THE TERTIARY STRUCTURE OF THE CA-ATPASE OF SARCOPLASMIC RETICULUM. Diana J. Bigelow and Giuseppe Inesi. University of Maryland School of Medicine; Dept. Biological Chemistry; Baltimore, MD.

Maleimide-directed spectroscopic probes bound to the Ca-ATPase were localized by resonance energy transfer efficiencies derived from donor (1) lifetime measurements (in both the time and frequency domains) as well as (2) steady state quantum yields. 2-(4'-maleimidylanilino)naphthalene-6-sulfonic acid (ANS-mal), 4-dimethylaminophenylazophenyl-4'-maleimide, and fluorescein-5'-maleimide modify cysteines of the A_1 tryptic fragment and show identical labeling characteristics to those of N-ethyl maleimide, shown previously to modify cysteines 344 and 363, bracketing the enzyme's phosphorylation site (Saito-Nakatsuka, et al., J Biochem., 1987, 101, 365). Energy transfer from ANS-mal to acceptors modifying the B tryptic fragment: (1) FITC at the nucleotide site and (2) iodoacetamid-directed probes (at Cys670 & Cys674) situated distal to FITC on this fragment, confirm a trigonal arrangement of the cytoplasmic domains of the ATPase. Energy transfer measurements from ANS-mal to the lanthanide, praseodymium (Pr^{3+}), indicate that at least one maleimide is located relatively close to the binding site(s) of this calcium analog. Pr^{3+} is shown to be a good analog for calcium binding to the high affinity sites on the enzyme since it competitively displaces calcium, as evidenced by the reversal of the specific calcium-dependent intrinsic fluorescence signal and activation of ATPase activity, over the same narrow range in Pr^{3+} concentration where energy transfer is observed. A model will be discussed relating these to previous measurements using site-directed probes.

M-Pos76 RAPID CALCIUM RELEASE ORIGINATES FROM A CALCIUM IONOPHORE-INACCESSIBLE SPACE IN THE TERMINAL CISTERNAL SARCOPLASMIC RETICULUM. László G. Mészáros[a] and Noriaki Ikemoto[b,c] a, II Inst. Biochem., Semmelweis Univ., Budapest, Hungary; b, Dept. Muscle Res., Boston Biomed. Res. Inst., Boston, Mass. 02114; c, Dept. Neurol., Harvard Med. Sch.

We have recently shown that the Ca^{2+} ATPase in the terminal cisternal SR (TC-SR) and that in the longitudinal tubular SR (LT-SR) show different kinetic properties (Mészáros and Ikemoto, Biophys. J. 51, 400a, 1987). The time course of Ca^{2+} uptake is biphasic in the TC-SR, while it is monophasic in the LT-SR, as determined by stopped flow spectrometry with arsenazo III and rapid filtration. The addition of Ca^{2+}-ionophores (A23187 or ionomycin) abolishes Ca^{2+} uptake in the LT-SR and the slow uptake phase in the TC-SR, while it has virtually no effect on the rapid uptake phase of the TC-SR. This suggests that the Ca^{2+} taken up in the rapid phase (10-15 nmole/mg protein) is occluded in somewhere within the TC-SR. Upon application of various Ca^{2+} release-inducing methods (e.g. caffeine, quercetin, trifluoperazine, and a Ca^{2+} jump) to the TC-SR vesicles after carrying out the Ca^{2+} uptake reaction in the presence of an ionophore, the Ca^{2+} pooled in an ionophore-inaccessible space was released rapidly. The release was blocked by ruthenium red. These results suggest that upon induction of release, the releasable Ca^{2+} originates from the ionophore-inaccessible, or occluded, space in the TC-SR. (Supported by grants from AHA, NIH and MDA).

M-Pos77 Transformation of sarcoplasmic reticulum Ca^{2+} ATPase isozymes in indirectly stimulated rabbit muscles.

K. Mabuchi, A.O. Jorgensen*, J. Fish*, F. A. Sreter, and J. Gergely. Dept. of Muscle Res., Boston Biomedical Research Institute; Boston, MA, Depts. of Neurology and Anesthesia, Mass. General Hosp.; and Depts of Biol. Chem. and Mol. Pharmacol., and Anesthesia, Harvard Med. School, Boston MA; 02114, * Dept. of Anatomy, University of Toronto, Ontario, Canada.

During the transformation of rabbit skeletal mucles from fast-twitch to slow-twitch, the switch from expressing the gene of type 2B myosin to the expression of type 2A myosin precedes the switch from type 2A to slow myosin (see Mabuchi et al., this meeting). We examined the expression of sarcoplasmic reticulum (SR) Ca^{2+} ATPase during such transformation by indirect stimulation (40Hz, 2.5 sec tetanus and 7.5 sec resting) using two monoclonal antibodies (McAbs), each reactive with either fast type or slow (cardiac) type SR Ca^{2+} ATPase (Jorgensen et al., Cell Motil. Cytoskel. 9, 164, 1988). The number of fibers reacting with anti-slow McAb was clearly increased after 6 days of stimulation in the core region of tibialis anterior. In general none of these fibers failed to react with anti-slow myosin McAb. This fact suggests that activation of genes coding for slow type myosin and slow type SR Ca^{2+} occurs in a coordinated fashion. At later stages of stimulation when more than 90% of the fibers had become pure type 1, as judged by reaction with the anti-myosin McAbs, many fibers still reacted with the anti-fast SR Ca^{2+} ATPase McAb. This may indicate that complete transformation with respect to SR Ca^{2+} ATPase never occurs. Examination of normal slow muscles showed the presence of many fibers reacting with both anti-slow- and anti-fast- SR Ca^{2+} ATPase McAbs. These observations suggest differences in the regulation of myosin and SR Ca^{2+} ATPase expression. (Supported by grants from NIH (HL 5949) and MDA).

M-Pos78 MODULATION OF SARCOBALL ANION CHANNEL VOLTAGE DEPENDENCE BY PERMEANT ANIONS.

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Single anion-selective channels from frog skeletal muscle SR were recorded using the sarcoball technique (Stein and Palade, Biophys. J. 54:357). The voltage dependence of the open probability (P_o) was found to be dependent on the concentration of permeant anions on either side of the patch membrane. With 50 mM or greater permeant anions present on both sides of the membrane, the P_o *vs* voltage plot yielded a bell-shaped curve centered around 0 mV. When permeant anions in the bath (Cl^-) were replaced with relatively impermeant anions (gluconate, MOPS, propionate or HEPES), the P_o *vs* voltage relationship was shifted by about -35 mV. Similarly, analogous experiments with the pipette solution produced a shift of comparable magnitude, but opposite polarity (+35 mV). A hypothesis, based on the existence of fixed positive charges (amino groups) lining the channel vestibules, was suggested to explain the shifts in voltage dependence. We evaluated this hypothesis by attempting to modify the fixed positive charges with several amino-group reagents and with increases in bath pH. The stilbene derivative DIDS reproduced the shift in voltage dependence, and other amino group modifiers (TNBS, SPIT) reduced the single channel conductance. These data offer some support for amino group involvement in the shifts in voltage dependence, and more strongly support the notion that amino groups are involved in conduction as well. The data also indicate that amino groups involved in conduction are separate from those related to voltage sensitivity.

M-Pos79 FURA-2 MONITORING OF THE EFFECT OF HALOTHANE ON CA^{2+} UPTAKE IN MEMBRANE VESICLES FROM MALIGNANT HYPERTHERMIC AND NORMAL PIG SR

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The effects of the anesthetic halothane on ATP-dependent Ca^{2+} uptake by membrane vesicles prepared from normal and malignant hyperthermic (MH) pig skeletal muscle were compared. The fluorescence of micromolar concentrations of Fura-2, added outside the vesicles, was used to continuously monitor the decline in $[Ca^{2+}]_{free}$ due to active uptake of Ca^{2+} by the oxalate loaded vesicles. The uptake velocity ($d[Ca^{2+}]_{total}/dt$) was calculated from the $[Ca^{2+}]_{free}$ vs time data with the Kd of the Fura-2:Ca^{2+} complex. Kinetic parameters for the Ca^{2+} uptake were compared for vesicles from both normal and MH pig skeletal muscle SR as a function of [halothane] present in the uptake buffer. Calcium transport by both the normal and MH vesicles exhibited cooperativity with respect to free calcium concentration (Hill coefficient ~2). Both normal and MH vesicles showed a similar concentration dependent decrease in calcium transport as a function of increasing halothane. However, this inhibition was only observed at halothane concentrations greater than those produced during anesthesia. This method of using the Ca^{2+} indicator Fura-2 as a high affinity, low capacity Ca^{2+} buffer permits the continuous monitoring of calcium uptake and accurate determination of kinetic parameters with as little as 20ug of membrane protein. Supported by NIH HL41188

M-Pos80 EFFECTS OF AMINO BINDING REAGENTS ON THE CALCIUM PLUS MAGNESIUM DEPENDENT ATPASE ACTIVITY OF FAST SKELETAL MUSCLE SARCOPLASMIC RETICULUM.

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Aminophospholipids are required for restoration of maximum activity to the delipidated SR of fast twitch skeletal muscle. All phospholipids have the ability to restore activity, but only in the presence of PE is activity maximized. We reacted purified SR with the amino cross-linking reagent 1,5-difluoro-2,4-dinitrobenzene (DFDNB) and amino binding reagent 2,4,6-trinitro-benzene sulfonic acid (TNBS). In both cases ATPase activity was inhibited drastically above 0.25mg reagent/ mg SR protein, TNBS inhibiting the activity almost 100% and DFDNB inhibiting the activity 70 - 75%. Inclusion of ATP or azide in the reaction medium, prior to the addition of DFDNB or TNBS preserved most of the ATPase activity. We have determined, using DFDNB, which phospholipids remain associated with the calcium pump protein following organic extraction and/or detergent treatment. Crosslinking of a specific peptide region is demonstrated.

Supported by NIH Grant HL 13870

M-Pos81 **RAPID FILTRATION MEASUREMENTS OF Ca^{2+}-INDUCED CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM OF NORMAL AND MALIGNANT HYPERTHERMIC PIG SKELETAL MUSCLES.**

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Calcium release from sarcoplasmic reticulum (SR) was studied using the "Rapid Filtration Technique". SR vesicles were isolated from normal (N) and malignant hyperthermic (MH) pig skeletal muscles, and passively loaded (at 1 mg of protein/ml) with 5-10 mM $^{45}Ca^{2+}$. Ca^{2+}-induced calcium release and the effects of known effectors (ATP, caffeine, and halothane) were investigated. 1) In the absence of ATP, extravesicular Ca^{2+} triggered calcium release within seconds and maximal efficiency was reached in the Ca^{2+} concentration range of 1 to 3 µM free Ca^{2+}. Maximal rate constants of calcium release were 0.4 and 1.2 s^{-1}, for N and MH SR, respectively. In the presence of 5 mM caffeine, the maximal rate constants of calcium release were shifted to 0.7 and 1.7 s^{-1}, and in presence of 0.01 % halothane (v/v), the maximal values were 0.7 and 4.5 s^{-1}, for N and MH SR, respectively. 2) In the presence of 1 mM ATP, extravesicular Ca^{2+} induced rapid calcium release (within 200 ms); further, there was a 10-fold increase in the rate constants of calcium release, and maximal values were 9.5 and 12 s^{-1} for N and MH SR, respectively. Under these conditions, halothane 0.01 % (v/v) or caffeine 5 mM did not change the maximal rate constants of calcium release; however, they induced a 2-fold increase of the calcium efflux rate constants in the low Ca^{2+} concentrations range.

All types of calcium release investigated with the rapid filtration technique have higher rate constants in MH than in N pig SR vesicles. (Supported by the AFM and the MDA).

M-Pos82 MECHANISM OF Ca^{2+} RELEASE FROM SARCOPLASMIC RETICULUM (SR) OF SLOW AND FAST TWITHCH SKELETAL MUSCLE- RYANODINE BINDING STUDIES. Young Sup Lee and Do Han Kim. Dept. of Medicine, University of Connecticut Health Center, Farmington, CT 06032.

Previously, we showed that slow twitch skeletal SR had a significantly lower rate of Ca^{2+} release activated by 2 uM Ca^{2+}, 2 mM caffeine and/or ryanodine (0.01-10 uM) than fast twitch skeletal SR suggesting that the longer twitch duration in the slow twitch skeletal muscle may be partly due to a different Ca^{2+} release mechanism (Lee et al. Biophys. J. 53, 136a, 1988). To examine the Ca^{2+} release mechanism in the slow SR, we have characterized ^{3}H-ryanodine binding to both types of SR under a variety of experimental conditions. In both types of SR, addition of 1 uM ruthenium red completely inhibited ^{3}H-ryanodine binding, whereas 2 mM caffeine or 50 uM doxorubicin activated ^{3}H-ryanodine binding 1.3 - 2.5 fold indicating that the Ca^{2+} release channel opened by caffeine or doxorubicin had higher ryanodine binding. Maximal ryanodine binding in the slow SR (2.9 pmol/mg) was singnificantly lower than that of fast SR (7.9 pmol/mg), whereas no significant difference was found in K_d (20 nM) and $[Ca^{2+}]$-dependence (peaked at 5 uM). Activation of ryanodine binding by increased pH (6.8 to 8.4) was significantly higher in slow SR (7.8 fold) than in fast SR (2.7 fold). These results suggest that the lower rate of Ca^{2+} release in slow SR is due in part to a differently regulated ryanodine-sensitive Ca^{2+} release channel in the slow SR. Supported by NIH Grant HL-33026 and Grant-in-Aid from AHA-Connecticut Affiliate.

M-Pos83 LANTHANIDES ELUCIDATE MULTIPLE STEPS INVOLVED IN CALCIUM ACTIVATION OF THE SARCOPLASMIC RETICULUM Ca-ATPase. Thomas C. Squier, Diana J. Bigelow, and Giuseppe Inesi. University of Maryland School of Medicine; Dept. of Biological Chemistry; Baltimore, Maryland 21201.

Calcium binding to the high affinity sites of the Ca-ATPase is accompanied by changes in the quantum yields of both (1) tryptophan residues (Δ F-TRP) located in helices associated with the bilayer and (2) covalently bound FITC (Δ F-FITC) near the nucleotide site, which accurately reflect the affinity and cooperative character of calcium binding. The ability of these spatially distant residues to sense calcium occupancy of the high affinity sites suggests the presence of a global conformational change associated with calcium activation. The involvement of the transmembrane helices in mediating calcium activation is suggested by both (a) the increase in anisotropy of the tryptophan residues upon calcium binding (Gryczynski et al., Biochemistry, 1988, in press) and (b) the ability of nonsolubilizing concentrations of detergent to interfere with both Δ F-TRP and Δ F-FITC. The process of calcium activation was further investigated through the use of Pr^{+3} and La^{+3}, which are good calcium analogs for the high affinity sites since they can competitively displace calcium and bind cooperatively to the ATPase, as well as support phosphoenzyme formation from ATP. However, we find that ΔF-FITC is preserved upon binding of these lanthanides, whereas ΔF-TRP is not, suggesting that lanthanides do not fully couple the conformational changes associated with calcium activation. This is interpreted as arising from multiple conformational steps resulting from calcium binding, where the greater affinity of the lanthanides to the entry sites prevents subsequent conformational changes associated with the transmembrane portion of the Ca-ATPase.

M-Pos84 THE EFFECTS OF FREE $[Mg^{2+}]$ ON RABBIT CARDIAC SARCOPLASMIC RETICULUM Ca^{2+} DEPENDENT ATPASE ACTIVITY. Stephen M. Krause, Department of Physiology, Jefferson Medical College, Philadelphia, PA 19107.

Recently it has been demonstrated that in skeletal muscle, sarcoplasmic reticulum (SR) ATPase activity is very sensitive to the free $[Mg^{2+}]$. Since recent studies using Mg^{2+} sensitive microelectrodes and NMR have indicated that the free $[Mg^{2+}]$ in the cardiac cell is closer to 0.4 mM than 3.2 mM as previously reported, we decided to investigate whether a lower free $[Mg^{2+}]$ altered the Ca^{2+} sensitivity of the SR ATPase. Cardiac SR was isolated from rabbit hearts in the presence of 25 µM PMSF by differential centrifugation. Maximal oxalate supported Ca^{2+} transport studies indicated a Ca^{2+} uptake rate of 0.529 $\pm$ 0.016 µmol Ca^{2+}/mg/min. Total ATPase activity was 1.279 $\pm$ 0.091 µmol Pi/mg/min during transport. The Ca^{2+} sensitivity of the ATPase was determined by direct measurement of ATP hydrolysis using a coupled enzyme assay over a free $[Ca^{2+}]$ of 0.1 to 10 µM at both 0.4 and 3.2 mM free Mg^{2+}. At free $[Mg^{2+}]$ 0.4 mM, maximal activation occured at 1 µM free Ca^{2+}. At a free $[Mg^{2+}]$ of 3.2 mM there was a shift in the maximal activation to 3.2 µM free $[Ca^{2+}]$. The EC_{50} was shifted from 0.48 µM to 0.91 µM free Ca^{2+}. The maximal ATPase activity was not significantly different; 1.012 $\pm$ 0.082 and 1.045 $\pm$ 0.042 µmol Pi/mg/min at a free $[Mg^{2+}]$ of 0.4 mM and 3.2 mM, respectively. In addition, at a free $[Mg^{2+}]$ of 0.4 mM, there was an inhibition of SR ATPase activity at free $[Ca^{2+}]$ greater than 1.5 µM. These results indicate 1) cardiac SR ATPase is very sensitive to the free $[Mg^{2+}]$ and 2) under Ca^{2+} overloading conditions, an inhibition of Ca^{2+} transport may occur resulting in an increase in diastolic intracellular $[Ca^{2+}]$.

M-Pos85 FUNCTIONAL COUPLING OF CREATINE KINASE WITH SARCOPLASMIC RETICULUM Ca^{2+}/Mg^{2+}-ATPase

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In muscle a significant amount of energy is spent for Ca^{2+}-sequestration by the Ca^{2+}/Mg^{2+}-ATPase of the sarcoplasmic reticulum (SR) that plays a crucial role in Ca^{2+} homeostasis. The phospho-creatine shuttle model (1) would predict that creatine kinase forms a functionally coupled compartment with the Ca^{2+}/Mg^{2+}-ATPase at the SR and thus be involved as an in situ ATP regenerator in supporting Ca^{2+}-pumping. Highly purified SR fractions were prepared from chicken pectoralis muscles and analysed for the presence of CK. Vesicles derived from longitudinal SR, contained 0.803 ± 0.455 I.U. of CK per mg of SR protein. The SR-bound CK was identified as muscle-type MM-CK. Immunogold localization showed labeling on the outside of the SR vesicles by anti-CK antibodies. To study the role of SR-bound CK, the velocity of calcium pumping into SR vesicles was measured using Antipyrylazo III. The results show that the amount of CK bound to the SR vesicles, representing a minimal value due to loss of CK during purification, was sufficient to support 24% of Ca^{2+}-pumping of SR vesicles in vitro in the presence of CP and ADP compared to maximal pumping in the presence of ATP. Thus, CK bound to SR in vivo may be physiologically significant for regeneration of ATP used by the Ca^{2+}-ATPase and for regulation of local ATP levels in the proximity of the Ca^{2+}-pump involving direct functional coupling of the two enzymes.

1) Wallimann T., Eppenberger H.M. (1985) Cell & Muscle Motility 6:239-285 (J.W.Shay ed) Plenum Press

M-Pos86 PHOSPHORYLATION OF THE CALCIUM-TRANSPORTING ATPase BY La·ATP: RAPID PHOSPHORYL TRANSFER PRECEDED BY A RATE-LIMITING CONFORMATIONAL CHANGE. A.M. Hanel and W.P. Jencks.

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The calcium-transporting ATPase of rabbit sarcoplasmic reticulum preincubated with 40 uM added exterior calcium is phosphorylated by 300 uM ATP and 250 uM $LaCl_3$ with an observed rate constant of 6.5 s^{-1} (pH 7.0, 25 C, 40 mM MOPS, 100 mM KCl). The phosphoenzyme (EP) formed in 5 s from 10 uM [γ-^{32}P]ATP and 250 uM $LaCl_3$ reacts biphasically with ADP in the presence of labeled ATP. The initial rapid loss of EP ($k_{obs} \geq 1000$ s^{-1}) is indicative of rapid phosphoryl transfer to and from the enzyme. The burst is followed by a slower first-order approach to equilibrium between phosphorylated and ATP-bound enzyme species. Both the fraction of EP disappearing in the burst and the observed rate constant of the slow phase increase proportionally with increasing [ADP] having maximal values of 0.34 and 64 s^{-1}, respectively, at saturating ADP ($K_{1/2}$ = 0.22 mM). The rate constant for ATP dissociation from E·Ca_2·La·ATP (≤2 s^{-1}) is too slow to account for the loss of EP at 64 s^{-1} following the burst. The addition of unlabeled ATP with 200 uM ADP does not change either the burst size or the rate of EP disappearance. These results necessitate that the slow phase of of the reaction with ADP is the equilibration of a reaction step not involved in either phosphoryl transfer or ATP dissociation. That step, which is rate limiting in both directions, is the conformational change interconverting the ^{c}E·Ca_2La·ATP intermediate to the form of the enzyme that is activated for phosphoryl transfer, ^{a}E·Ca_2La·ATP (Petithory and Jencks, 1986 Biochemistry 25, 4493). Lanthanum, as the catalytic ion, slows the conversion of ^{c}E to ^{a}E from 220 s^{-1}, observed with Mg^{2+}, to 6.5 s^{-1}. However, the actual rate of phosphoryl transfer remains too fast to measure.

M-Pos87 EFFECT OF LIPID LATERAL PHASE SEPARATION ON THE Ca^{+2}ATPase OF THE SARCOPLASMIC RETICULUM. F. Asturias, D. Pascolini, K. Blasie, Dept. of Chemistry, U. of Pennsylvania, Phila., PA 19104

Our previous meridional and equatorial X-ray diffraction studies established the existence of a temperature induced structural transition in the sarcoplasmic reticulum (SR) Ca^{+2}ATPase which occurs at a temperature corresponding to the [Mg^{+2}]-dependent upper characteristic temperature (t_A) for lipid lateral phase separation (LPS) in the SR membrane. Furthermore, the functionality of the ATPase was also found to be linked to t_A: at temperatures below t_A, the lifetime of the first phosphorylated conformation, E_1 ~P, was found to be considerably extended. We have now studied the effect of water activity on LPS in the SR membrane, and the effect of water activity and [Mg^{+2}] on LPS behavior of bilayers of isolated SR lipids. LPS was observed in the SR lipid bilayers and the LPS behavior was found to be qualitatively similar to that observed for the intact SR membrane. Namely, LPS occurs only at low water contents, independently of the [Mg^{+2}]. At low water contents, changes in [Mg^{+2}] have the same effect on LPS (t_A is slightly higher for higher [Mg^{+2}]), and t_A values for both systems coincide to within a few degrees. However, LPS is more extensive for the isolated lipids than for the intact membrane, especially at high [Mg^{+2}]. As previously found for the SR membrane, LPS in the isolated lipid bilayers is fully reversible, and more extensive for samples partially dehydrated at temperatures below t_A. This could explain why E_1 ~P transient trapping is more efficient in SR membranes partially dehydrated at low temperature, which also show the largest change in the structure of the enzyme. The observation that LPS behavior for the SR membrane and the isolated lipids (no protein) are qualitatively similar strongly supports the conclusion that the LPS behavior of the SR membrane lipids is the driving force behind the observed conformational change in the ATPase, and the resulting increase in E_1 ~P lifetime. Supported by NIH HL-18708

M-Pos88 PERTURBATIONS IN THE LIPID PHASE OF SCALLOP FRAGMENTED SARCOPLASMIC RETICULUM.

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Phase changes in scallop fragmented sarcoplasmic reticulum (FSR), having the Ca^{2+}-ATPase subunits in different states were examined with steady state fluorescence intensity of the solid lipid phase probe trans-parinaric acid (tPA) over the temperature range $^{-}3°-37°C$. The states examined were: $E_1(Ca^{2+})_2$; E_2P (P1 lattice); E·Mg·AMP-PCP (P2 lattice); and E·Mg·P-P-P (P2 lattice). Phase changes in the overall lipid phase were detected using direct excitation of tPA at 320nm and perturbations in the vicinity of lipid-protein interface were observed using indirect excitation of tPA via energy transfer from tryptophyl side chains excited at 250 nm. The perturbation temperatures depended upon the state of Ca^{2+}-ATPase. Phase changes detected using direct excitation were as follows: $E_1(Ca^{2+})_2$, 23°C; 34°C; E_2P, 11°C; E·Mg·AMP-PCP, 17.5°C; E·Mg·P-P-P, 18.5°C. Perturbations in the lipid-protein interface were observed only with the Ca^{2+}-ATPase in the $E_1(Ca^{2+})_2$ and E_2P forms at about 11°C. This phenomenon correlates well with the discontinuity on the Arrhenius plot of Ca^{2+}-ATPase activity which occurs at the same temperature. (Kalabokis, V.N., and Hardwicke, P.M.D. (1988) J. Biol. Chem., in press).
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M-Pos89 HALOTHANE ACTIVATES THE CALCIUM RELEASE CHANNEL OF THE SARCOPLASMIC RETICULUM FROM FROG.

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We report here that halothane, a volatile anesthetic that is able to trigger episodes of malignant hyperthermia (MH) in susceptible animals and patients, activates the calcium release channel from frog (Caudiverbera caudiverbera) sarcoplasmic reticulum (SR), by increasing channel fractional open time (Po) without change in channel conductance. Highly purified SR membrane vesicles were fused into POPE:PS:PC = 5:3:2 planar bilayers. 37 mM Ba was used in the intrareticular compartment as the current carrier; Hepes was used as impermeant anion. 100 μM EGTA or HEDTA were used in the cytosolic compartment as Ca buffers.

Halothane was added to the cytosolic compartment (final concentration 10 to 150 μM). Halothane activation was strongly calcium dependent. At pCa 6.0, 13 μM halothane increased Po from 0.33 ± 0.10 (mean ± S.D.) to 0.78 ± 0.13. This was mainly due to a large decrease in the closed time constants (τ_{slow}: 44.4 to 9.3 ms and τ_{fast}: 9.6 to 1.6 ms) and a slight increase in the slow open time constant (from 13.5 to 20.4 ms). 26 μM halothane further increased Po to 0.97 ± 0.01. Similar increases in Po could be elicited at pCa 6.5, whereas no effect was seen at pCa 7.0. The calcium dependence of halothane activation suggests a possible explanation for the triggering of MH episodes during general anesthesia, as higher resting levels of free intracellular calcium have been found in skeletal muscle fibers from susceptible patients (1) (normal patients = 0.11 μM, MH patients = 0.38 μM) and pigs (2).

1. López et al. Muscle Nerve 8: 355-358, 1985. 2. López et al. Muscle Nerve 9: 85-86, 1986.
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M-Pos90 MEMBRANE ALTERATIONS AFTER FUSION OF ISOLATED SARCOPLASMIC RETICULUM.

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Data from liposome systems have shown that increasing radius of curvature can have profound effects on the dynamics of membrane phospholipids. To determine if similar constraints might operate in biological membranes, fusion of a well-characterized membrane system was carried out. Isolated skeletal sarcoplasmic reticulum (SR)("light" fraction, 1.122-1.270 g/cm^3) was aggregated (dehydrated) for 1 hr. in 55% polyethylene glycol (8,000 m.wt.), rinsed in buffer to remove PEG followed by lyophilization and rehydration in buffer. Freeze fracture of these samples showed large (up to 2 microns dia. compared to 150 nm dia. native vesicles) unilamellar vesicles in which the Ca^{2+} ATPase intramembrane particles retained their native asymmetric distribution in the SR bilayer. Thin layer chromatography of TNBS-treated native and fused SR vesicles revealed a change from the native asymmetric bilayer distribution of phosphatidylethanolamine (PE) (65:35 outer:inner) to a symmetrical distribution (52:48) in the larger fused vesicles. While Ca^{2+}ATPase activity was not altered, the rate of Ca^{2+} uptake was lower in the fuse vesicles. These data suggest that vesicle curvature can effect biological membrane structure and function. Initial studies supported by HL13870.

M-Pos91 EVIDENCE FOR AN EXCHANGEABLE PHOSPHOLIPID ANNULUS IN ISOLATED SARCOPLASMIC RETICULUM. Roger J. Bick, Keith A. Youker, W. Barry Van Winkle and Mark L. Entman. Section of Cardiovascular Science, Dept. of Medicine, Baylor College of Medicine, Houston, Texas 77030.

When isolated sarcoplasmic reticulum vesicles (SR) are extracted with organic solvents or are "stripped" with the non-ionic detergent octaethylene glycol dodecyl ether ($C_{12}E_8$), not all the membrane phospholipids are removed from the calcium pump protein; the remaining phospholipid species differ markedly from native preparations. Either method of stripping results in a residual phospholipid complement made up primarily of PE (50%) and PS (30%); the latter originates in the inner monolayer and the former in the outer monolayer. Both PE and PS contain high percentages of unsaturated fatty acids and are highly plasmalogenic. Phospholipids remaining with the calcium pump protein after maximal stripping ("annular phospholipids"), are predominantly unsaturated, plasmalogenic PE and PS. The ratio of PE and PS in the annulus changes with turnover conditions (PE, 50 - 30% ; PS, 25 - 35%) suggesting that phospholipid association is conformation dependent. Non-specific phospholipid exchange protein has been used to show that although these lipids are specific in remaining associated with the CaATPase during subsequent stripping, they are exchangeable and therefore the apparent "phospholipid annulus" is not a rigid construct, but results from a functionally determined selective association between specific phospholipids and the CaATPase protein.

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M-Pos92 THE EFFECTS OF 2,3-BUTANEDIONE MONOXIME ON TWITCH FORCE AND STEADY STATE FORCE RELATIONSHIPS IN FERRET PAPILLARY MUSCLES. Tomoko Ohkusa and Judith K. Gwathmey. Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Diacetyl monoxime (BDM) is a negative inotropic agent. One proposed mechanism of action involves altered calcium handling by the sarcoplasmic reticulum. Another proposed mechanism of action involves an inhibitory effect of BDM at the level of the myofilaments. The following experiments were done to address this issue: 1) Peak isometric force versus $[Ca^{2+}]_o$ curves were constructed and compared to steady state force-$[Ca^{2+}]_o$ achieved by tetanization of intact papillary muscles. Experiments were performed after muscle exposure to ryanodine, a sarcoplasmic reticulum inhibitor. % Force versus $[Ca^{2+}]_o$ (0.5-24mM) curves were constructed in the presence and absence of BDM (3mM) for peak isometric force and steady-state force. A concentration of 3mM BDM was chosen because at this concentration, BDM does not affect the sarcolemma or action potential parameters (Li T. et al. J Pharmacol. Exp. Ther. 232,688-695, 1984). Exposure to ryanodine reduced peak isometric force by 66±8%. In the presence of ryanodine, at normal extracellular calcium concentrations, BDM further reduced peak isometric force by 60±6% and steady state force by 27±6% without affecting time course of the isometric twitch or tetanus. At extracellular calcium concentrations between 1-8 mM, BDM caused a significant reduction in peak isometric and tetanized force as well as a shift to the right in both force-$[Ca^{2+}]_o$ curves. At higher $[Ca^{2+}]_o$ (16-24mM) there was no significant difference in isometric or steady-state force (BDM effects could be antagonized). Our results suggest that alterations in SR Ca^{2+} handling cannot solely explain the effects of BDM. In addition BDM appears to have effects at the level of the myofilaments by: 1) altering Ca^{2+} sensitivity; 2) decreasing the maximum force generated.

M-Pos93 CAVULIN, A NOVEL MARKER OF TRANSVERSE TUBULES (TTs) AND CAVEOLAE (CAV) IN RABBIT SKELETAL MUSCLE. A. O. Jorgensen*, W. Arnold*, A. C.-Y. Shen*, M. Gaver+ and K.P.Campbell+, *Department of Anatomy, University of Toronto, Toronto, Canada; +Department of Physiology and Biophysics, University of Iowa, Iowa City, USA.

We have recently reported that the α_1 subunit (170k Da) of the 1,4-dihydropyridine receptor (DHPR) is confined to TT and CAV in rabbit skeletal muscle (Jorgensen et al, 53:469a, 1988). To further characterize the protein composition of TTs, mAb IXE112 prepared against purified rabbit TTs was tested for its ability to label TT by immunofluorescence staining of cryostat sections from rabbit diaphragm muscle. The results showed that the intensity of labeling of fast fibers was only slightly higher than that of slow fibers. Specific labeling in longitudinal sections was confined to the A-I interface of the fibers where the TTs are localized, but apparently absent from the sarcolemma (SL). Immunoelectron microscopical labeling of cryofixed, freeze-dried rabbit psoas muscle showed that specific labeling was confined to TT and CAV, but absent from the lateral portion of the SL. Specific labeling of other membrane-bound organelles and of myofibrils was not observed. Immunoblotting of skeletal membrane fractions with mAb IXE112 showed that this mAb specifically binds two polypeptides of 120k and 24k Da which are enriched in isolated TT vesicles. The relationship between these two polypeptides is being investigated. The results show that mAb IXE112 defines a novel component of rabbit skeletal muscle which like the DHPR, is a specific marker of TT and CAV. We have named this component cavulin. The function of this novel protein is presently unknown.

M-Pos94 BIOGENESIS OF TRANSVERSE TUBULES IN RABBIT SKELETAL MUSCLE.
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We have previously shown that a novel protein, cavulin, defined by mAb IXE112, is a specific marker of transverse tubules (TTs) and caveolae in rabbit skeletal muscle. To study the biogenesis of TTs, the temporal appearance and distribution of cavulin was determined in developing rabbit skeletal muscle during the period of de novo TT formation. Specific labeling of transverse cryosections was first observed in developing skeletal fibers on day 17 of gestation. At this time, specific labeling was confined to distinct foci and short rod-like structures at the periphery of the myofibers. On day 24, specific labeling was still most prominent at the cell periphery but the distinct foci and rod-like structures extended further towards the center of the fibers. In addition, the rod-like structures were occasionally observed to branch. In myofibers of 1-2 day old rabbits, specific labeling was present throughout the cytosol and distributed in a chicken-wire-like network. Specific labeling of cavulin at all the developing stages studied was absent from the sarcolemma. The results presented are consistent with the idea that cavulin is directly incorporated into the forming TT at the time of their de novo formation, rather than being incorporated into the SL and then subsequently transfered to forming TT.

M-Pos95 EVIDENCE FOR DISTINCT MOLECULAR COMPOSITION OF SMOOTH MUSCLE CAVEOLAE AND SARCOLEMMAL MEMBRANE
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We have recently (Jorgensen et al, these proceedings) used mAb IXE112 to identify a novel protein cavulin in rabbit skeletal muscle and demonstrated by immunoelectron microscopy that cavulin is confined to transverse tubules and caveolae in this tissue. To determine whether cavulin is also present in smooth muscle, cryosections from a variety of smooth muscle tissues were examined by immunofluorescence labeling with mAb IXE112. The results showed that a cavulin-like component was localized in distinct foci and strand-like structures at the periphery of arterial smooth muscle fibers. The intensity of labeling was approximately inversely proportional to the diameter of the vessels, i.e. the arterioles were most intensely labeled. Specific labeling was apparently absent from veins and non-vascular smooth muscle. Immuno electron microscopical studies of the basilar artery showed that the cavulin-like component is confined to caveolae and absent from the lateral portion of the sarcolemma. In conclusion. the results presented demonstrated for the first time that caveolae of arteral smooth muscle contain at least one component distinct from that of the lateral portion of the sarcolemma.

M-Pos96 MOLECULAR DYNAMICS OF PHOSPHOLIPID BILAYERS IN THE LIQUID-CRYSTALLINE STATE FROM DEUTERIUM NMR SPECTROSCOPY. Michael F. Brown*, Steven W. Dodd, Ulf Henriksson+, Amir Salmon, and Olle Soderman#. *Department of Chemistry, University of Arizona, Tucson, AZ 85721, U.S.A., +Department of Physical Chemistry, Royal Institute of Technology, Stockholm, Sweden, and #Division of Physical Chemistry 1, University of Lund, Sweden.

Measurement and analysis of the spin-lattice relaxation rates (R_{1z}) of phospholipid bilayers in the liquid-crystalline state can yield knowledge of their molecular dynamics over a range of different time-scales. We have conducted R_{1z} studies of multilamellar dispersions and small sonicated vesicles of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) with perdeuterated and specifically deuterated acyl chains. A total of nine magnetic fields were employed, corresponding to Larmor frequences ω_0 ranging from 2.5 MHz to 61.4 MHz. The R_{1z} rates can be approximated by an $\omega_0^{-1/2}$ dependence; fits to ω_0^{-1} or two-step Lorenztian dispersions are less satisfactory. Profiles of the R_{1z} rates of DMPC as a function of the deuterated chain segment position exhibit a square-law dependence on the corresponding order profiles. The relaxation may involve fast motions, including trans-gauche isomerizations and rotational diffusion of the chains, in addition to slower motions due to collective bilayer disturbances (order-director fluctuations). Work sponsored by NIH Grants GM41413, EY03754, and RR03529 and by the Swedish Natural Science Foundation.

M-Pos97 DISATURATED PHOSPHATIDYLCHOLINES IN THE LIQUID-CRYSTALLINE STATE STUDIED BY DEUTERIUM NMR SPECTROSCOPY. Steven W. Dodd and Michael F. Brown (Intr. by S. S. Berr). Department of Chemistry, University of Arizona, Tucson, AZ 85721.

Deuterium (^2H) NMR studies of the influences of the acyl chain and head group substituents of membrane phospholipids can yield information regarding the balance of forces influencing their properties in the liquid-crystalline state. We have continued our ^2H NMR studies of 1,2-diacyl-sn-glycero-3-phosphocholines containing identical saturated chains ^2H-labeled by perdeuteration: di(per-^2H-n:0)PC, where n = 12,14,16,18. Multilamellar dispersions were investigated and the ^2H NMR spectra were de-Paked to obtain oriented subspectra with increased resolution. Essentially complete profiles of the segmental order parameter S_{CD} as a function of chain position have been obtained at a series of different temperatures. The results show that the "plateau" in the order profiles increases in length as the number of acyl chain carbons increases. The additional mass due to an increase in the number of acyl chain carbons apparently leads to an increase in the bilayer thickness 2<L>, rather than the cross-sectional area per chain <A>. It is likely that <A> is governed by a balance of attractive and repulsive forces acting near the membrane water-hydrocarbon interface, which modulate indirectly the interactions between the chains. Work sponsored by NIH Grants GM41413, EY03754, and RR03529.

M-Pos98 POLYUNSATURATED PHOSPHATIDYLCHOLINES IN THE LIQUID-CRYSTALLINE STATE STUDIED BY DEUTERIUM NMR SPECTROSCOPY. Amir Salmon and Michael F. Brown (Intr. by T. P. Trouard). Department of Chemistry, University of Arizona, Tucson, AZ 85721.

Polyunsaturated phospholipid bilayers are of interest with regard to their possible roles in biological functions. Deuterium (^2H) NMR studies of a series of 1-acyl-2-docosahexaenoyl-sn-glycero-3-phosphocholines in the liquid-crystalline state have been carried out, in which the saturated sn-1 chain is perdeuterated, whereas the sn-2 polyunsaturated (22:6) chain is protiated: (per-^2H-n:0)(22:6)PC where n = 12,14,16,18. Powder-type ^2H NMR spectra were obtained and de-Paked to yield oriented subspectra with increased resolution. Compared to disaturated PC's, ^2H NMR spectra of the mixed-chain polyunsaturated PC's have additional intermediate quadrupolar splittings, which increase in number as the number of saturated acyl chain carbons is increased. Profiles of the segmental order parameter S_{CD} as a function of chain position were derived. Using a simple diamond-lattice model, the effective lengths <L> of the saturated chains projected along the bilayer normal and one-dimensional thermal expansion coefficients α were derived. The saturated acyl chains of the mixed-chain polyunsaturated bilayers have greater configurational freedom, in the liquid-crystalline state, than in disaturated phosphatidylcholine bilayers. Work sponsored by NIH Grants GM41413, EY03754 and RR03529.

M-Pos99 DEUTERIUM NMR SPECTROSCOPY OF POLYUNSATURATED AND DISATURATED PHOSPHATIDYLCHOLINE LIPID BILAYERS. Theodore P. Trouard, Robin L. Thurmond, Judith A. Barry, Stuart S. Berr, Amir Salmon, Steven W. Dodd, S.C. Shekar, and Michael F. Brown. Department of Chemistry, University of Arizona, Tucson, AZ 85721.

The unique properties of polyunsaturated phospholipids, as compared to their saturated counterparts, may play vital roles in membrane functions. ^{2}H NMR studies have been conducted of randomly oriented, multilamellar dispersions of two homologous series of phosphatidylcholines with perdeuterated acyl chains: di(per-^{2}H-n:0)PC and (per-^{2}H-n:0)(22:6)PC, where n = 12,14,16,18. Detailed comparisons of the moments of the lineshapes as a function of temperature were carried out for each lipid to determine the liquid crystalline (L_α) to low temperature phase transition temperatures. The results extend into the low temperature phase previous studies of the L_α phase, which reveal interesting differences in the physical properties of disaturated and polyunsaturated PC's (1). The effects of chain length asymmetry and unsaturation on the chain ordering within the low temperature phase were examined. The moments were also used to test the hypothesis that a Law of Corresponding States is applicable to these systems. (1) A. Salmon et al., J. Am. Chem. Soc. 109, 2600-2609 (1987). Supported by Postdoctoral Fellowships from the NIH (J.A.B.) and Am. Heart Assoc. (S.S.B.), an NSF Predoctoral Fellowship (A.S.), and by NIH Grants GM41413, EY03754, and RR03529.

M-Pos100 **APPLICATIONS OF FOURIER TRANSFORM RAMAN SPECTROSCOPY TO HIGHLY FLUORESCENT MEMBRANE ASSEMBLIES: POLYENE ANTIBIOTIC / DIPALMITOYLPHOSPHATIDYLCHOLINE INTERACTIONS.**
E. Neil Lewis and Ira W. Levin, Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892.

For the study of biological samples conventional dispersive Raman spectroscopy using visible laser excitation usually suffers from a dominant fluorescent background originating from either the presence of sample impurities or the intrinsic nature of the preparation. A potentially promising method for overcoming the problems of fluorescence involves the relatively new technique of Fourier transform Raman spectroscopy which couples a near-infrared laser excitation source to a Michelson interferometer. However, major impediments to the further development of the technique for biological studies remain those of accurate sample temperature control and of measurement within the constraints imposed by fragile biological systems subject to excitation wavelengths with a high degree of thermal character. Data are presented demonstrating the use of fiber optic assemblies to minimize laser induced sample heating. The use of water library spectra in conjunction with spectral matching techniques are used as a non-invasive means of estimating sample temperatures. As an example of the utility of Fourier transform Raman spectroscopy in obtaining spectra of highly fluorescent biological samples, we examine the thermotropic behavior of multilamellar lipid assemblies in the presence of polyene antibiotics; namely, amphotericin B, amphotericin A and nystatin. In particular, amphotericin B and amphotericin A show the ability to induce the interdigitated gel phase in aqueous dispersions of dipalmitoylphosphatidylcholine.

M-Pos101 **COMPARISON OF LINOLENIC (18:3ω3) AND DOCOSAHEXAENOIC (22:6ω3) ACIDS IN PHOSPHOLIPID BILAYERS.** William Stillwell*, Daniel Belcher*, William Ehringer* and Stephen R. Wassall#, Departments of Biology* and Physics#, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223

Omega-3's are believed to be involved in prevention of a number of human afflictions. Two of these fatty acids are linolenic acid (18:3) and docosahexaenoic acid (DHA, 22:6). DHA is particularly abundant in marine oils and is the fatty acid most closely studied for its beneficial effects on health. Although the mode of action for ω-3 fatty acids is not known, one suggestion is that they are incorporated into membranes and there provide some specific function. Here we distinguish between the effects of linolenic and DHA on the membrane properties of permeability, fusion and fluidity. The fatty acids were tested as both free fatty acids as well as mixed chain 18:0, 18:3 and 18:0,22:6 phosphatidylcholines. By both isotonic erythritol swelling and carboxyfluorescein release, DHA is seen to enhance PC permeability to a larger extent than linolenic acid. Both free fatty acids similarly increase lipid vesicle fusion as monitored by fluorescence. With the probe DPH, both fatty acids are shown to decrease fluorescence anisotropy (increase fluidity) in saturated 18:0, 18:0 PC gel state bilayers. The fatty acids broaden and decrease the phase transition temperature of this phospholipid. In the liquid crystalline state, linolenic acid has no effect on these bilayers while DHA decreases membrane fluidity. Similar results were seen with unsaturated 18:1, 18:1 PC bilayers in the liquid crystalline state. Using a series of fluorescent anthracene stearic acid probes, 18:0, 18:3 PC demonstrates a decrease in bilayer fluidity relative to the 18:0,22:6 PC at positions 2, 6, 9 and 12 in the chain.

M-Pos102 FLUORESCENCE DEPOLARIZATION STUDY OF LAMELLAR LIQUID CRYSTALLINE TO INVERTED CYLINDRICAL MICELLAR PHASE TRANSITION OF PHOSPHATIDYLETHANOLAMINE by K.H.CHENG and S.Yang, Physics Department, Texas Tech University, Lubbock, TX

The orientational order and rotational dynamics of fluorescent phospholipid DPH-PC embedded in Dioleoylphosphatidylethanolamine (DOPE) were studied by fluorescence depolarization technique. Upon increasing the temperature, the calculated wobbling diffusion constant D of the probe was found to decrease at the lamellar (L) to inverted cylindrical (H) phase transition (10°C). This suggested that the increased gauge rotamers of the alkene chains in the H phase imposes a constraint in the wobbling motion of the fluorophore. The calculated ratio of order parameter in the L phase to that in the H phase was 1.7 and different from the theoretical value of 2.0 as predicted from the change in packing symmetry. The lower experimentally observed order parameter ratio indicated a slightly higher local order packing of the fluorophore in the H phase as a result of the reduced surface curvature of the lipids in the H phase. The perturbation of this L - H phase transition by cholesterol and phosphatidylcholine was also examined. Similar to the effect in L phase, cholesterol significantly increases the order packing of the lipids in the H phase. A substantial decrease in rotation of the fluorophore in the H phase was also noticed. The L - H phase transition for the PE/PC mixture occurred only at temperature higher than 45°C and at PC content less than 12.5 %. Intermediate phase was predicted from the symmetry consideration for lower temperature and higher PC content.

M-Pos103 DETERMINATION OF THE LATERAL DISTRIBUTION OF PYRENE-PC IN LIPID BILAYERS.
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The lateral distribution of 1-palmitoyl-2-[(10-pyrenyl)decanoyl] phosphatidylcholine (PyrPC) in lipid bilayers at various temperatures and pressures has been determined by using the ratio of excimer fluorescence intensity to monomer fluorescence intensity (E/M). At low PyrPC concentration, the E/M ratio is proportional to the PyrPC concentration. The nonlinearity appearing at higher probe concentration (>10 mol%) is attributed to the clustering of the PyrPC molecules in lipid bilayers. When an excited PyrPC molecule is not in a PyrPC cluster the excimer formation is a relatively slow diffusion limited process. However, when an excited PyrPC molecule is located within a cluster of PyrPC molecules the probability of excimer formation is larger. This makes understandable the positive deviation of E/M ratio from the linearity towards higher pyrene concentrations. A theoretical model has been developed, by generalizing the model proposed by Birks et al. (Proc. R. Soc. London, Ser. A. 1963, 275, 575), for the case of high probe concentrations. By comparing the experimentally obtained concentration dependence of the E/M ratio with the theoretically predicted E/M function, valuable information about the lateral distribution of PyrPC in lipid bilayers has been obtained. It is found that the lateral distribution varies with both temperature and pressure. Supported by the U.S. Army Research Office and the EI of AHA and CIBA-GEIGY.

M-Pos104 **STUDY OF N-ACYLPHOSPHATIDYLETHANOLAMINES IN AQUEOUS DISPERSION BY INFRARED AND RAMAN SPECTROSCOPIES**

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The effect of the headgroup chain length on the structure and on the thermotropic behavior of N-acyldipalmitoylphosphatidylethanolamines (N-acyl-DPPE) has been studied by infrared and Raman spectroscopies. The results show that the N-acyl-DPPE's can be devided in two classes depending on the N-acyl chain length. For N-C4 to N-C8 DPPE's, the N-acyl chain is too short to penetrate deeply into the bilayer and remains at the level of the glycerol backbone of the lipid. This results in a significant lowering of the gel to liquid crystalline phase transition of the lipid and to the formation of intermolecular hydrogen bonds between the amide groups. For N-C10 to N-C16 DPPE's, the N-acyl chain is anchored into the bilayer. Since the N-C10 chain is shorter than the palmitoyl chains of the lipid, it increases the conformational disorder in the bilayer and lowers the cooperativity of the phase transition. On the other hand, the spectral features of N-C16-DPPE and DPPE are almost identical, even though the phase transition of the N-acyl lipid is slightly lower in temperature. Both classes of N-acyl-DPPE's form lamellar phases of hexagonal symmetry and the rotation of the lipid chains in the liquid crystalline phase is hindered by the presence of the N-acyl group. In addition, the disruption of the hydrogen bonds between the amino and phosphate groups in the N-acyl-DPPE's results in an increase of the hydration of the phosphate group compared to DPPE.

M-Pos105 THE MECHANISM OF CONCENTRATION DEPENDENT PHOSPHOLIPID TRANSFER. Jeffrey D. Jones and Thomas E. Thompson (sponsored by Ronald P. Taylor). Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908.

We have previously demonstrated that spontaneous phospholipid transfer between bilayer vesicles is characterized not only by a first order desorption rate, but, at higher vesicle concentration, by a second order process dependent on vesicle concentration (Jones and Thompson, Biochemistry, in press). We have extended our studies to examine the mechanism of this second order process. The first and second order rate constants have been determined for the transfer of 1,2-dimyristoyl phosphatidylcholine and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) between POPC vesicles at 30°C. The ratio of the first to the second order rate constant was found to be identical in both systems. This result indicates that vesicle-vesicle interactions act to increase the rate of spontaneous monomer desorption from the vesicle bilayer. The temperature dependence of POPC transfer was determined between 27° and 50°C. The Arrhenius activation energy for first order process was found to be approximately 50 kJ/mole. However, the ratio of the first and second order rate constants was found to be virtually independent of temperature These results suggest a model in which the rate of lipid desorption from vesicle bilayers is enhanced in transient vesicle-vesicle complexes. Transfer was determined by measuring the movement of tritium labelled lipids between vesicles which were chromatographically separated at appropriate time intervals. (Supported by PHS-NIH Grants GM-14628 and GM-23673).

M-Pos106 BILAYER STABILIZATION ACTIVITY OF PHOSPHATIDYLGLYCEROL IN DIOLEOYLPHOSPHATIDYLETHANOLAMINE LIPOSOMES. Ana Tari and Leaf Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

Although dioleoylphosphatidylethanolamine (DOPE) aggregates into the hexagonal phase at room temperature and neutral pH, different amphipathic compounds can be used to stabilize the bilayer phase of DOPE. A systematic study of the effects of (i) acyl chain length, (ii) degree of unsaturation and (iii) number of acyl chains of the stabilizer on the stabilization activity was done, using phosphatidylglycerol (PG) as a model stabilizer. DOPE, either alone or mixed with various PG's at different mole %, were sonicated to form unilamellar vesicles. Stability of liposomes was evaluated by entrapping calcein, a self-quenching fluorescent dye. Stable liposomes would have a higher degree of fluorescence-quenching than unstable (or leaky) liposomes. An active stabilizer would stabilize the DOPE liposomes at a low mole %. For the acyl chain length effect, saturated PG's were compared and the relative activity of stabilization followed the order: C14 $\geq$ C16 > C12 >>C18. For C18 PG's, unsaturated ones showed a higher activity than the saturated one. At the same mole % of either C14 or C16 PG's, double chain PG's stabilized liposomes whereas lyso PG's did not. Preparing stable liposomes entrapping calcein requires that the bilayer phase of DOPE is stabilized and, furthermore, that the stabilized bilayer is not phase separated. The latter effect might be the reason for the lack of the stabilization activity in some PG's. We are currently investigating the possibility using Differential Scanning Calorimetry. (Supported by NIH grants CA 24553 and AI 25843).

M-Pos107 THE ROLE OF CHOLESTEROL IN THE STABILITY OF pH-SENSITIVE, LARGE UNILAMELLAR LIPOSOMES PREPARED BY DETERGENT DIALYSIS METHOD. Dexi Liu and Leaf Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

Large unilamellar liposomes prepared by an octylglucoside-dialysis method were examined for stability at 37°C in the presence or absence of human plasma, using the release of the entrapped calcein as a fluorescence marker. The liposomes were acid-sensitive as they were composed of dioleoyl phosphatidylethanolamine, oleic acid and cholesterol. The stability of the liposomes in the absence of plasma was significantly enhanced with increasing cholesterol content. However, the maximal calcein release at pH 5 decreased linearly with increasing cholesterol content of the liposome, indicating that cholesterol had reduced the acid-sensitivity of the liposomes. In the presence of human plasma, calcein release exhibited a biphasic behavior with a fast (plasma-sensitive) and a slow (plasma-resistant) component. Inclusion of cholesterol in the liposomes resulted in an increased proportion of the plasma-resistant component. Liposomes pretreated with human plasma, after removal of excess plasma and the released calcein by gel filtration, showed a remarkable stabilty both in the presence and absence of human plasma. This result indicated that the plasma-resistant component was in fact significantly stabilized by the treatment of plasma. The acid sensitivity of the plasma-treated liposomes was the same as that of the untreated ones. These results are discussed in terms of the interactions of two kinetically distinct processes: a fast destabilization reaction and a slow stabilization reaction. Supported by NIH grants CA 24553 and AI 25834.

M-Pos108 STABILIZATION OF SMALL UNILAMELLAR, pH-SENSITIVE LIPOSOMES BY PROTEIN COMPONENTS IN HUMAN PLASMA. Dexi Liu and Leaf Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

Small unilamellar liposomes prepared by sonication containing calcein and composed of dioleoylphosphatidylethanolamine (DOPE) and oleic acid (OA) were incubated in the presence of human plasma at 37°C. The release of calcein was about 15% in plasma up to 8 hours incubation comparing with a greater release (about 70%) in PBS at the same conditions. Instead, DOPC:OA liposomes at the same conditions release about 70% in plasma and only 10% in PBS. Total release of calcein of DOPE:OA liposomes was observed in BSA solution and the release was completely blocked by preincubation of liposome with plasma. A few protein bands shown by SDS-polyacrylamide gel electrophoresis indicates that proteins are associated with liposomes after incubation with plasma. The results of 2D TLC analysis indicates that lipids in plasma are not involved in liposome stabilization. The incubation time course showed that the stabilization process is very fast. Human serum and dialyzed human plasma have the same effect on stabilization, indicating that components involved in clotting and small molecular weight components in plasma are not involved. A floating centrifugation assay with liposomes containing [^{14}C] OA suggested that about 40% of oleic acid were extracted from liposomes after 1 hour incubation with human plasma at 37°C. These results suggested that the liposomes composed of DOPE:OA are stabilized by the protein components of the human plasma and that the composition of the liposome is altered after interaction with the human plasma. Supported by NIH grants CA 24553 and AI 25834.

M-Pos109 PROPERTIES OF BILAYER LIPID MEMBRANES FORMED FROM LIQUID CRYSTALS. Zdzislaw Salamon and H. Ti Tien, Membrane Biophysics Laboratory (Giltner Hall), Department of Physiology, Michigan State University, East Lansing, MI 48824

The conformations of lipids in crystals and biomembranes have much in common. In fact, the structure of a lipid bilayer is frequently depicted as a liquid crystal in two dimensions. In this communication we report a new type of bilayer lipid membrane (BLM) with aqueous interfaces which are formed from thermotropic liquid crystals (nematic-6CB, smectic-8CB, and cholesteric-cholesteryl palmitate, ChP). The electrical and photoelectrical properties of these BLMs have been investigated. We suggest that BLMs of this type consist of two molecular layers with a smectic-like structure. Incorporation of compounds such as mesotetraphenylporphyrin (TPP) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) impart photosensitivity to the membrane. The photocurrent across the double layers affects membrane properties which can be seen in the BLM capacitance changes. It is shown that a TCNQ-cyanobiphenyl charge transfer complex formed in the BLM is responsible for the observed photochanges. The possible mechanism of photoinduced electrical effects in these types of BLMs will be discussed.

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References:

Liquid Crystals, 3, 169 (1988).
Mol. Cryst. Liq. Cryst., 154, 195 (1988).
Photochem. Photobiol., 48, 281 (1988).

M-Pos110 LATERAL MOBILITY OF LIPIDS IN POLYMERIZED LANGMUIR-BLODGETT FILMS. Fei Wang, S. Q. Xu, Janos H. Fendler, Robert R. Birge, and Bennie R. Ware. Graduate Biophysics Program and Department of Chemistry, Syracuse University, Syracuse, New York, 13244.

The goal of the research presented here is to develop stabilized lipid bilayers with restricted lateral mobility for the construction of stable planar models for biological membranes and for the construction of molecular electronic devices. Fluorescence photobleaching recovery (FPR) has been used to measure the lateral mobilities of fluorescent-labeled lipid molecules in a matrix of polymerizable lipids. The magnitude of the translational diffusion coefficient is correlated with the degree of polymerization of the bilayer. Polymerization was induced by UV irradiation of lipids containing C=C bonds in either the head group or the hydrocarbon chain. Illumination was carried out both on the lipid monolayer formed on the aqueous subphase of a Langmuir trough and on the LB film after deposition onto a solid substrate. Translational diffusion coefficients were observed to decrease in proportion to the total photon dose of irradiation. However, for a constant total photon dose, lower illumination intensities, which are known to produce polymer chains of greater length, also produced reduced lateral mobility. Further decrease of lipid mobility could be induced by the inclusion of a photopolymerizable counterion in the subphase.

M-Pos111 RAMAN SPECTROSCOPIC STUDIES OF THE PACKING PROPERTIES OF MIXED DIHEXADECYL- AND DIPALMITOYL-PHOSPHATIDYLCHOLINE BILAYERS. Mark T. Devlin and Ira W. Levin, Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892

X-ray diffraction studies suggest the existence of two separate gel phases for mixed dihexadecylphosphatidylcholine (DHPC)/dipalmitoylphosphatidylcholine (DPPC) bilayers.[1,2] For one gel phase the lipid chains are interdigitated, while the other gel phase exhibits the conventional bilayer form. We use Raman spectroscopy to provide a detailed molecular analysis of the intermolecular and intramolecular interactions of the DHPC and DPPC molecules within these mixed bilayers. Observation of the methylene chain C-H stretching modes of DHPC and the methylene chain C-D stretching modes of DPPC-d_{62} for various mixed DHPC/DPPC-d_{62} bilayers enables the packing characteristics and conformational order of each lipid to be monitored separately. The spectral data indicate that the packing properties of DPPC-d_{62} in the mixed lipid bilayers remain relatively unchanged, while the intramolecular and intermolecular properties of DHPC change dramatically as a function of the composition of the DHPC/DPPC-d_{62} mixed bilayer. This is a consistent with a model based upon the existence of three domains for the mixed lipid system; namely, a pure DPPC-d_{62} domain, a pure DHPC domain and an interface or boundary region between the two bulk domains.

1. Kim, J.T., Mattai, J., & Shipley, G.G. (1987a) *Biochemistry 26*, 6599-6603.

2. Lohner, K., Schuster, A., Degovics, G., Muller, K., & Laggner, P., (1987) *Chem. Phys. Lipids 44*, 61-70.

M-Pos112 DETERMINATION OF MEMBRANE SURFACE CHARGE BY COLLOIDAL CRYSTAL TECHNIQUES.
Joel A. Cohen, H. Daniel Ou-Yang, and Paul M. Chaikin; Univ. of the Pacific, San Francisco, CA; Lehigh Univ., Bethlehem, PA; Princeton Univ., Princeton, NJ; Exxon Res. & Eng. Co., Annandale, NJ.

The traditional method for measuring membrane surface charge, particle electrophoresis, is of limited utility due to the generally unknown location of the hydrodynamic shear surface relative to the particle (membrane) surface. Further hydrodynamic complexities arise if the surface is not smooth. By forming colloidal crystals, one obtains an electrostatically-stabilized, equilibrium system in which the above hydrodynamic uncertainties are eliminated. Whereas in particle electrophoresis one measures mobilities of individual non-interacting particles, with colloidal crystals one measures bulk properties of a strongly-interacting particle suspension. Since the interaction potential is known (screened Coulomb repulsions), the average particle charge is calculable from bulk parameters. Colloidal-crystalline suspensions were made from unilamellar PG/PC vesicles formed in deionized water by high-pressure extrusion through Nuclepore filters. Dynamic light scattering showed mean vesicle diameters of 0.1 um and polydispersities of ±40%. Static light scattering and freeze-fracture electron microscopy indicated the presence of ordered vesicular arrays. The vesicle charge was determined by measurement of (1) shear modulus, (2) dynamic light scattering at forward scattering angle, which is related to osmotic compressibility, (3) effective "pH" of the suspension, (4) osmotic pressure, (5) Donnan/Nernst potential of the suspension relative to a standard H^+ buffer, measured across a proton-permeable membrane (gramicidin-treated BLM). These techniques can also be used, within limits, to measure ion-membrane binding. (Supported in part by NIH GM35241.)

Phospholipid and polystyrene colloidal crystals will be on display.

M-Pos113 THE ELECTROSTATIC PROPERTIES OF THE PHOSPHOINOSITIDES: A DISCRETENESS-OF-CHARGE EFFECT WITH PIP_2.
M. Langner[1], S. Marcelja[2], D. Cafiso[3], S. McLaughlin[1]. [1]Dept. of Physiology & Biophysics, HSC, SUNY, Stony Brook, NY; [2]Dept. Applied Mathematics, ANU, Canberra, Australia; [3]Dept. Chemistry, University of Virginia, Charlottesville, VA.

We studied the effect of PI and PIP_2 on the adsorption of counterions and coions to vesicles using fluorescence and ESR techniques described elsewhere (Biochemistry, 1986, 25: 8206 & 8214). Increasing the mole % of the monovalent lipid PI in PC/PI vesicles enhanced the adsorption of counterions (cationic probes) and depressed the adsorption of coions to the vesicles. We can describe these results using the Boltzmann relation and the average surface potential obtained from electrophoretic mobility measurements. The results are also consistent with the predictions of the Gouy-Chapman-Stern theory,which assumes the charges are smeared uniformly over the surface. The effect of the trivalent lipid PIP_2 on the adsorption of the coions and counterions to PC/PIP_2 vesicles, however, could not be described by the Boltzmann equation using the potential determined from mobility or electrode measurements; both coions and counterions adsorbed more strongly than predicted. The deviations were larger for coions than counterions. We compared our results with the predictions of a modern statistical mechanical theory that takes into account the mobile discrete nature of the surface charge, correlation effects between the ions in the diffuse double layer, the finite size of these ions, and image charge effects. The predictions of this theory agreed with the experimental results obtained with both PC/PI and PC/PIP_2 vesicles.

M-Pos114 FLUORESCENT PIP_2, TRANSFER OF PIP_2FROM MICELLES TO BILAYERS, AND ELECTROSTATIC LIPID-PROTEIN INTERACTIONS.
Robert V. McDaniel & Stuart McLaughlin, HSC, SUNY Stony Brook, NY 11794-8661

We synthesized a pyrene derivative of phosphatidylinositol 4,5-bisphosphate, $PyPIP_2$. Control experiments suggest the headgroup of PIP_2 is intact and the acyl chains are labelled with about one pyrene/PIP_2. Specifically, $PyPIP_2$/PC and PIP_2/PC vesicles have the same electrophoretic mobility; neomycin affects the zeta potential of these vesicles in an identical manner; $PyPIP_2$ and PIP_2 comigrate on a TLC plate; phosphate analysis and optical density measurements indicate that $PyPIP_2$ has a phosphorous/pyrene ratio of about 3:1. We are studying the transfer of $PyPIP_2$ from micelles to membranes. When we mixed $PyPIP_2$ micelles with spin labelled PC vesicles, we observed quenching of the pyrene fluorescence, which suggests that $PyPIP_2$ became incorporated into the bilayers and that it might be possible to increase the PIP_2 content of biological membranes by exposing them to PIP_2 micelles. (PIP_2 micelles also increased the nonactin conductance of PC planar bilayers and imparted a negative electrophoretic mobility to PC multilamellar vesicles, but the interpretation of these experiments is less straightforward.) We are also studying the interaction of trivalent anionic $PyPIP_2$ lipids with cationic membrane-bound peptides; $PyPIP_2$ is useful because it quenches the fluorescence from tryptophan residues in peptides.

M-Pos115 THE EFFECT OF ELECTRIC FIELDS ON THE CONFORMATION AND ORDER OF ALIGNED PHOSPHOLIPID DISPERSIONS STUDIED BY SOLID STATE NMR. P.A. Osman and B.A. Cornell, CSIRO, P.O.Box 52, North Ryde, N.S.W. 2113, Sydney, Australia.

Dispersions of phospholipid aligned on metal coated glass coverslips have been observed by carbon-13, deuterium-2 or nitrogen-14 solid state NMR whilst being subjected to electric fields of up to 10,000,000 volt/metre. The application of electric fields of this magnitude is achieved by applying voltages of order 10-100 volts across coverslips separated by 3-5μm. Difficulties encountered with the technique included the prevention of electrical contact between adjacent metal surfaces, electrolytic effects at the metal to solution interface and the maintenance of the homogeneity of the dispersion between the coverslips. At the electric fields achieved in these studies the conformation of the polar region of the phospholipid is essentially unaltered. The high dielectric constant for this region causes an attenuation of the local electric field. Significant effects have been observed associated with the olefinic groups within the hydrocarbon region of the bilayer. Further studies will be reported of the effects of electric fields on solute molecules bearing dipole moments.

M-Pos116 MOLECULAR ORDER IN ALIGNED LIPID SYSTEMS STUDIED BY SOLID STATE CARBON-13 NMR. V.L.B. Braach-Maksvytis, F. Separovic and B.A. Cornell, CSIRO, P.O. Box 52, North Ryde, NSW 2113, Sydney, Australia.

A qualitative description of the average conformation of Lα phase phosphatidylcholine has been deduced from the carbon-13 reduced chemical shift anisotropies obtained from aligned bilayer systems (Braach-Maksvytis and Cornell, 1988).

This presentation will discuss the quantitative description of the conformation based on order parameters. Following the analysis of deuterium NMR data by Doane (1979), the method has been extended to a description of a general molecular fixed axis set, different to the symmetry frame of a uniaxial phase for an axially asymmetric carbon-13 shielding tensor. The rigid lattice tensor elements and orientations have been obtained from model compounds. The head group and backbone regions will be compared between different classes of lipid in terms of the quantitative description of the average conformation obtained from the order parameter analysis.

Braach-Maksvytis, V.L.B., and B.A. Cornell, 1988. Biophys. J. 53:839-843.
Doane, J.W., 1979. NMR of Liquid Crystals. In Magnetic Resonance of Phase Transitions. F.J. Owens, C.P. Poole, and H.A. Farach, editors. Academic Press, Inc., NY. 171-246.

M-Pos117 SCALING OF THE ^{2}H-NMR ORDER PARAMETER DISTRIBUTION ALONG THE PHOSPHOLIPID CHAINS: CORRELATION BETWEEN THE MAGNITUDE AND THE SHAPE OF THE ORIENTATION ORDER PROFILE IN THE L_α PHASE.
Michel Lafleur*, Bernard Fine+, Colin P.S. Tilcock*, Pieter R. Cullis* and Myer Bloom+. * Department of Biochemistry and + Physics Department, University of British Columbia, Vancouver, B.C., V6T 1W5.

A new method has been developed to determine the complete order profile from a single powder spectrum of a phospholipid which has a palmitoyl chain fully deuteriated. After dePakeing the powder spectrum, the intensity is normalized to the number of deuterium nuclei present on a chain and a quadrupolar splitting is assigned for every CD_2. Assuming a monotonic decrease of the order parameter S(n) as a function of the carbon position (n) from the one nearest to the interface to the end of the chain, the profile is then determined.

Various factors influence the order profile. A decrease of the temperature or the addition of cholesterol restrict the motion along the chain. The values of the order parameters are also larger for POPE than for POPC, for the same temperature. This indicates a tighter packing of the acyl chains of POPE, probably due to the smaller area occupied by the POPE polar head group.

For the wide range of order parameters obtained in the L_α phase of POPC and POPE when temperature and cholesterol concentration are varied, two striking features have been observed:

a) If $S_i(n)=S_j(n)$ for any single value of n, then $S_i(n)=S_j(n)$ for all values of n.

b) If $S_i(n)\neq S_j(n)$, the fractional difference is larger near the end of the chain.

However, the L_α to H_{II} phase transition induces a drastic change of the order profile. The plateau which is characteristic of the bilayer is replaced in the hexagonal phase by a sharper decrease of S(n) as a function of n. This is related to the change of the geometrical space available for the motion of the chain during this phase transition.

M-Pos118

THE LONG-RANGE "HYDROPHOBIC" INTERACTION CAN BE EASILY EXPLAINED BY CORRELATED FLUCTUATIONS OF ADSORBED IONS. Rudi Podgornik, Donald C. Rau and V.Adrian Parsegian, National Institutes of Health Bethesda, MD 20892. Intr. by John J. Kasianowicz.

Eyebrows were no doubt raised six years ago by the assertion that"The hydrophobic interaction is long range, decaying exponentially with distance". Two papers described a force with a ≈ 11 nm decay length extending nearly to 10 nm separations between macroscopic curved mica surfaces made non-polar by the adsorption of amphiphiles. A progression of later reported papers described attractive forces of ever longer range and decay lengths. Most recently, attractions extending to 90 nm separation and 13 to 16 nm decay rates further strain the traditional interpretation of these particular "hydrophobic" interactions in terms of solvent perturbation by the non-polar surfaces. Given the popularity of the "hydrophobic effect" to explain a wide variety of biological, colloidal and interfacial phenomena, a force of such strength and range has attracted much attention. We propose an alternate explanation based on the correlation in the density of surface adsorbed ionic charge. The range and the decay of these forces emerges directly from the screening lengths of the dilute ionic solutions in which measurements are usually made. The principal equations of our theory can be stated briefly. One considers the surface of adsorbed ions as a layer having a density that can fluctuate with desorption into or adsorption from the solution. In the model there are two plane parallel surfaces separated by a distance h with intervening electrolyte of Debye length $1/\kappa$. On each surface there is adsorption/desorption of ions of both signs. We shall suppose that the correlations among the adsorbing charges along the same surface are of correlation length ξ, therefore the total interaction free energy between the two surfaces can be obtained exactly to give $\Delta F(h) = kT\,\pi Ei(-2\kappa h)/\xi^2$ where Ei(x) is the exponential integral. We have been able to fit three different types of data, including cases with known and unknown (conductivity water) ionic strength to the above form of the interaction free energy, obtaining in all cases reasonable values for the parameters.

M-Pos119 CURVATURE, LIPID SEGREGATION, AND THE HEXAGONAL-LAMELLAR TRANSITION FOR PE/PC MIXTURES IN WATER AND TETRADECANE. R. P. Rand, N. L. Fuller, Brock University, St Catharines, Canada, S. Gruner, Princeton University, Princeton, N. J. and V. A. Parsegian, NIH, Bethesda, MD.

Amphiphiles respond to polar and non-polar solvents. X-ray diffraction and osmotic stress have been used to examine the phase behavior, and both the dimensions and the work of deforming the aqueous cavities of the structures formed by DOPE/DOPC mixtures as a function of concentration of the two solvents, water and tetradecane (td). In the absence of td, given adequate DOPC, only lamellar phases form in excess water, all of which become single reverse hexagonal (H_{II}) phases in excess water and td. The picture which emerges suggests that the spontaneous radius of curvature, R_o, of lipid monolayers, is expressed in the H_{II} phase, is allowed by the relief of hydrocarbon chain stress by td, and increases with the ratio of DOPC/DOPE. Very large R_o's, with water contents higher than the La phase in the absence of td, are understood in terms of curvature energy in monolayers adding to hydration of the polar groups. Initial work of removing water goes into changing monolayer curvature rather than dehydrating polar groups. Structural dimensions of single H_{II} phases forced to deviate from R_o by osmotic stress indicate that as the size of the water cylinder decreases (a) the molecular area is compressed and expanded at the polar and hydrocarbon ends of the molecule respectively, (b) either the degree of hydrocarbon chain splaying and compression are different for molecules aligned in different directions around the water cylinder if those cylinders are circularly symmetrical or deviation from circular symmetry of the water cylinders exists. For the lipid mixtures in the absence of sufficient td or water, the resultant enforced deviation of the H_{II} monolayer from R_o in the single H_{II} phase that forms initially is sufficiently powerful to cause demixing of the phospholipids into coexisting La and H_{II} phases. The rate of demixing and the dimensions of the resultant phases suggest that the DOPE/DOPC ratio of the H_{II} phases is adjusted so that its R_o matches the amount of td available, i.e. that curvature energy is minimized.

M-Pos120 NON-ELECTROSTATIC FORCES BETWEEN BILAYER SURFACES OF METHYLATED AMINES. R. P. Rand, N. L. Fuller, Brock University, St Catharines, Canada, and V. A. Parsegian, NIH, Bethesda, MD.

In addition to electrostatic double-layer repulsion, charged bilayers experience shorter range forces at separations <~20 Å (Biochemistry 17:3163,1978). The difficulty of detecting these latter forces on top of electrostatic interactions explains why hydration repulsion has been seen most easily between neutral species. Yet this non-coulombic force between charged bilayers appears to be as large and as worthy of study as between neutral species. Using the osmotic stress method, we have measured forces between bilayers of long chain substituted amines, specifically dihexadecyldimethylammonium acetate in 5 mM to 500mM acetate solutions. For bilayers brought together in solutions of low salt concentration, there is a clear break at 11 Å separation from the exponential electrostatic double-layer repulsion. Below this separation there is instead an exponential repulsion with 1.6 Å decay. In high salt solutions there is an exponential repulsion of 2.7 Å decay for separations < 17 Å. That these shorter range interactions were not seen in previous measurements with lipids adsorbed to the crossed cylindrical mica sheets of a "surface force apparatus" (J.Phys.Chem.90:1637(1986)) can be explained by the opposite bilayer curvatures enforced in that system and the stress limitations caused by mica bending. Thus, including this molecule with a very small polar group, every lipid system yet investigated shows hydration repulsion at short range.

M-Pos121 Ca-INDUCED INTERACTION BETWEEN PS-CONTAINING BILAYERS ACTS TO DEHYDRATE NEIGHBOURING PC MOLECULES. J. Coorssen, N. L. Fuller and R. P. Rand, Brock University, St Catharines, Canada. (Intro. by P. Nicholls) All interacting phospholipid bilayers, including dioleoylphosphatidylcholine (DOPC), feel mutual hydration repulsion that dominates at separations > about 20-30 Å. But acidic phospholipids, including dioleoylphosphatidylserine (DOPS), triggered to interact by divalent cations attract so strongly as to exclude intervening water. What happens when hydration repulsion and Ca-induced attraction compete when mixed DOPS/DOPC bilayers are triggered to interact by addition of calcium? We have used X-ray diffraction, freeze-fracture em, density gradient centrifugation and osmotic stress to analyse the structures formed when large unilamellar vesicles of DOPS/DOPC interact in 10 mM $CaCl_2$. The repeat spacing of a single multilamellar structure is 51.4 Å from 0 to ~30 mole% DOPC, and increases systematically but with increasing disorder up to pure DOPC. Freeze fracture em indicates adhered but uncollapsed vesicles contribute to this disorder. Centrifugation yields, over the entire range of mixtures, a single band whose density systematically and sensitively decreases with DOPC content. We conclude that no bulk lipid segregation occurs as a result of the interaction. For up to 30 mole% DOPC, mild to extreme osmotic dehydration of these precipitates results in no change in the lamellar phase nor the appearance of any other structure. We conclude that up to ~30 mole% DOPC must be nearly completely dehydrated and contained in the single completely collapsed lamellar structure. This shows that the Ca-DOPS reaction can serve to dehydrate neutral, highly hydrated neighbours, but that the $Ca(DOPS)_2$ product formed (Florine and Feigenson, Biochemistry 26, 1757: Biophys J. 51, 537a) must be laterally segregated from DOPC within continuous bilayers. From 30-80 mole% DOPC hydration repulsion prevents the collapse, mild dehydration orders the system but maintains the bilayer separation, and stronger dehydration triggers the bilayer collapse, but now the lipids bulk segregate into two lamellar phases. These results show that the competition between hydration repulsion and Ca-induced attraction results in a rich heirarchy of interactions and structures, both stable and metastable.

M-Pos122 OBSERVATION OF DYNAMIC THICKNESS FLUCTUATIONS IN LIPID BILAYERS.
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We report the observation of spontaneous (thermally-generated) thickness fluctuations in a planar lipid bilayer. Such dynamic thickness fluctuations could have a significant effect on the interactions of polypeptides and proteins in bilayers, for example, on the formation and stability of the dimeric gramicidin A channel (Helfrich, P. and E. Jakobsson, Biophys. J. 53:327a, 1988, and Huang, H.W., Biophys. J. 50:1061-1070, 1986).

A laser beam was focused to a waist diameter of 6.5 μm on a monoolein/decane planar bilayer at room temperature, and the intensity of the specular reflection was monitored as a function of time. The power spectrum of the intensity fluctuations is directly proportional to the power spectrum of thickness fluctuations with wavelengths longer than the beam diameter. The observed intensity fluctuations indicate an rms amplitude of 0.1% for thickness fluctuations with wavelengths longer than 6.5 μm.

We have modeled the bilayer as a thin, incompressible liquid film. Continuum hydrodynamics yields a dispersion equation for the non-propagating thickness fluctuation disturbances in the film. The observed power spectrum is well fit by the hydrodynamic theory with a film viscosity η of 10 ± 1 mPa-sec (centipoise) and a thickness compressibility U" of 9 ± 1 J/mm^4. If the continuum theory is valid down to a wavelength of 10 nm, we predict an rms amplitude of thickness fluctuations of about 20% of the hydrocarbon core thickness. (Work supported by NSF grant DMB-8603426 and ACS/PRF grants 15927-B7 and 15928-B7.)

M-Pos123 ELECTRO-MECHANICAL PERMEABILIZATION OF LIPID VESICLES: ROLE OF MEMBRANE TENSION AND COMPRESSIBILITY, D. Needham and R. M. Hochmuth, Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC 27706.

A simple micropipet technique was used to determine the critical electric field strength for membrane breakdown as a function of the applied membrane tension for three different reconstituted membranes: SOPC, RBC lipid extract and SOPC:CHOL,1:1. For these membranes the elastic area expansivity modulus increases from ~200-600 dyn/cm, and the tension at lysis increases from 5.7-13.2 dyn/cm, i.e. the membranes become more cohesive with increasing cholesterol content. The critical membrane voltage, V_C, required for breakdown was also found to increase with increasing cholesterol from 1.1 - 1.8 V, at zero membrane tension. We have modelled the behavior in terms of the bilayer expansivity. Membrane area can be increased by either tensile or electrocompressive stresses. Both can store elastic energy in the membrane and eventually cause breakdown at a critical area dilation or critical energy. The model predicts a relation between tension and voltage at breakdown and suggests a shift to lower membrane tensions and critical membrane voltages with decreasing elastic modulus. We also show videomicrographs of adherent vesicle electrofusion which demonstrate that the critical electric field strengths for permeabilization and fusion are the same for bilayers at close (28 Å) separation and under weak (0.02 erg/cm^2) adhesion energy.

*SOPC = stearoyloleoylphosphatidylcholine, RBC = red blood cell, CHOL = cholesterol

M-Pos124 INTERACTIONS BETWEEN LIPID CHAINS AND NON-LIPID MOLEULES IN BILAYERS: A MONTE CARLO STUDY. H. L. Scott, S. Kalaskar, J. Xing, A Chowdhury, Department of Physics, Oklahoma State University, Stillwater, OK 74078

The Monte Carlo method has been utilized to perform a theoretical study of equilibrium hydrocarbon chain packing in a bilayer in the presence of a non-lipid molecule. The model system consists of an array of 99 lipid chains with one end attached to an interface. Near the center of this array is a single non-lipid molecule. Non-lipid molecules modeled in this study include cholesterol and gramicidin A. Within the array lipid chains are allowed to move laterally, rotate about their long axes, and undergo trans-gauche isomeric rotations. The coordinates of all non-hydrogen atoms for both cholesterol and gramicidin were hand entered into the programs. While the non-lipid molecules were not allowed translational or long-axis rotational motions, rotations were allowed in the side chains. Intermolecular interactions were calculated from 1-6-12 potential functions with interaction parameters taken from the literature. These interactions were summed over all pairs of atoms in all molecules within a cutoff radius. Calculations were carried out for cholesterol interacting with C-14, C-16, and C-18 chains, and for gramicidin A interacting with C-14 chains. The major results are presented as plots of chain segment order parameter profiles and as tabulated standard deviations in the averages. The results show that the rigid non-lipid molecules strongly hinder the abilities of the nearest neighbor chains to undergo gauche rotations. This does not mean that the neighbor chains are all in all-trans conformations. Rather, once initial rotations occur in these chains steric hindrances make further isomeric changes difficult. Supported by NSF Grant DMB 87-02644.

M-Pos125 MEMBRANE CONTACT, FUSION AND HEXAGONAL (H_{II}) TRANSITIONS IN PHOSPHATIDYLETHANOLAMINE LIPOSOMES. Theresa M. Allen, Keelung Hong and Demetrios Papahadjopoulos, Department of Pharmacology, University of Alberta, Edmonton, Canada T6G 2H7 and Cancer Research Institute, University of California, San Francisco, CA 94143, U.S.A.

The behavior of phosphatidylethanolamine (PE) liposomes has been studied as a function of temperature, pH, ionic strength, divalent cation concentration, lipid concentration and liposome size using techniques of differential scanning calorimetry (DSC), dynamic light scattering, assays measuring liposomal lipid mixing, content mixing and content leakage and by a new fluorimetric assay for detecting hexagonal (H_{II}) transitions in dilute solution. The results demonstrate that 1) the H_{II} phase does not occur in dilute unilamellar PE suspensions, even at temperatures which thermodynamically favor the H_{II} state in packed multilayers, in the absence of conditions leading to liposome aggregation. 2) Liposome aggregation leading to the formation of H_{II} phase can be induced by packing of the liposomes by addition of Ca^{2+} or Mg^{2+}, increasing $[Na^+]$ and lowering pH (increasing $[H^+]$). 3) Formation of the H_{II} phase is associated with high rates of leakage of entrapped contents and fusion of liposomes at H_{II} permissive temperatures is accompanied by rapid contents loss. 4) PE liposomes under non-aggregation conditions will exist in a bilayer non-leaky state at temperatures well above the H_{II} transition temperature. 5) PE liposomes can be induced to fuse below the H_{II} transition temperature without significant loss of entrapped contents. 6) There is no correlation between fusion and the formation of H_{II} phase.

M-Pos126 TRANSLATIONAL DIFFUSION AND FLUID DOMAIN CONNECTIVITY IN MIXED LIPID BILAYERS. Winchil L.C. Vaz, Eurico C.C. Melo, Thomas E. Thompson and Thomas M. Jovin. (Sponsored by Carl E. Creutz). Max-Planck-Institut für Biophysikalische Chemie, D-3400 Göttingen, F.R.G. and Department of Biochemistry, University of Virginia Medical Center, Charlottesville, VA 22908, U.S.A.

Fluorescence recovering after photobleaching (FRAP) was used to examine the translational diffusion of a lipid probe, NBD-dilauroyl phosphatidylethanolamine (NBD-DLPE), in multibilayers prepared from mixtures of dimyristoyl phosphatidylcholine (DMPC) and distearoyl phosphatidylcholine (DSPC). NBD-DLPE was chosen because of its preferential (>95%) partitioning into the fluid phase in bilayers with coexisting fluid and gel phase domains. The FRAP experiments yield information on the diffusion rate of the probe and the fractional recovery after photobleaching. The latter parameter allows conclusions regarding the degree of connectivity of the fluid domains in the mixed system where complete recovery after photobleaching is diagnostic of a fully connected fluid phase. The phase diagram for DMPC/DSPC mixtures shows a miscibility gap below the solidus line when the mole fraction of DSPC is less than 0.62. Fluid domain connectivity in mixtures of 0.20, 0.35, 0.50, 0.65, and 0.80 mole fraction of DMPC occurrs at temperatures of 28.4°, 36.0°, 44.0°, 47.2°, and 50.0°C, respectively, corresponding to gel phase amounts of 13, 26, 27, 34 and 74%, respectively. Models for the domain shape upon formation from a gel phase mixture are presented.

M-Pos127 STRUCTURE AND PROPERTIES OF C16:0 SULFATIDE AS A FUNCTION OF HYDRATION.
Kumkum Saxena, Pavanaram K. Sripada and G. Graham Shipley, Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

Following deacylation of bovine brain sulfatide under mild alkaline conditions and reacylation using palmitoyl chloride (Koshy and Boggs, Chem. Phys. Lipids 1983, 34, 41), N-palmitoyl galacto-sulfatide (NPGS) has been synthesized. By differential scanning calorimetry, anhydrous NPGS exhibits an endothermic transition Tm = 93°C (ΔH=5.5 Kcal/mol NPGS). With increasing hydration (50 mM sodium phosphate buffer, pH 7.0; 50 mM NaCl), Tm decreases reaching a limiting value of 49°C (ΔH=8.2 Kcal/mol NPGS) at ~ 20 wt% buffer. X-ray diffraction data have been recorded at 30 and 60% hydration below (20°C) and above (60°C) Tm. At 20°C, a sharp wide angle reflection in the $1/4.2Å^{-1}$ region indicates the presence of a gel phase, whereas at 60°C, a broad reflection at $1/4.4Å^{-1}$ characteristic of a melted chain L_α phase is observed. Lamellar diffraction patterns consistent with the presence of bilayer phases are observed at both temperatures but interestingly the bilayer periodicities in the gel phase (d ~63Å; 20°C) and the liquid crystal phase (d~ 60Å; 60°C) are insensitive to hydration. This suggests that the presence of ~130 mM Na^+ is sufficient to shield the charge contributed by the sulfate group and to prevent bilayer "swelling".

M-Pos128 IONIC AND NON-IONIC SOLUTES AFFECTS PHASE TRANSITIONS IN PHOSPHATIDYLETHANOLAMINE. Christina Aurell Wistrom, Lois M Crowe, John H. Crowe, Department of Zoology, Univ. of California, Davis, CA 95616.

Some phosphatidylethanolamines (PE) are known to form non-bilayer phases such as inverted hexagonal phase under physiological conditions. Many of the different non-bilayer phases have been associated with physical damage during freezing or dehydration to biological membranes containing high amounts of PE. Formation of pure lipid domains during chilling or freezing of cells may cause formation of lipid particles or lipid phase separation leading causing leakage of cell content or fusion of cell membranes. We have studied the thermotropic phase behavior of hydrated natural egg PE in the presence of ionic and non-ionic solutes using Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and electron microscopy. Neutral salts from the Hofmeister series as well as other salts, carbohydrates and certain amino acids at concentrations 0.1 - 1.0 M were used to hydrate the egg PE. DSC scans of hydrated eggPE showed that each specific solute had effects on both the main gel to lamellar phase transition and the lamellar to hexagonal phase transition. Solutes known to be water-structure breakers (chaotropic agents) such as NaSCN, urea, guanidine hydrochloride all lowered the main transition and increased the temperature of the hexagonal transition. Polar water-structure makers (kosmotropes), such as NaCl, divalent cations and carbohydrates all raised the main transition and lowered the transition temperature for the hexagonal transition. The additive effect of Hofmeister salts on PE could also be detected in changes of main and hexagonal phase transitions. The data here presented show similarities with Hofmeister effects already known from protein systems. (Supported by grant DMB 85-18194 from NSF).

M-Pos129 EFFECTS OF DIMETHYLSULFOXIDE ON STABILITY OF PHOSPHOLIPID BILAYERS. J.H. Crowe, T.J. Anchordoguy, J.F. Carpenter, and L.M. Crowe. University of California, Davis, CA 95616.

Despite the well known cryoprotective effects of dimethylsulfoxide (DMSO) on cells and tissues, this molecule is also known to be toxic at physiological temperatures. Based primarily on theoretical considerations Arakawa et al. (1988. Submitted ms) have recently proposed a mechanism for this toxicity. They have suggested that at elevated temperatures, when hydrophobic interactions are strengthened, DMSO shows a hydrophobic interaction with proteins. Under these conditions, the protein is denatured. We have now extended this suggestion to phospholipid bilayers. Sonicated vesicles of egg PC were prepared with carboxyfluorescein (CF) trapped in the aqueous interior. The vesicles were frozen in liquid nitrogen and retention of the CF was recorded. At low [DMSO] (0.05-20%) leakage of CF during freezing was completely inhibited. However, at [DMSO] >30% the vesicles leaked more than the controls. When the vesicles were heated in various [DMSO] they commenced leaking CF at <20 C in the presence of 50% DMSO. At lower concentrations, leakage occurred at higher temperatures, inversely proportional to [DMSO]. In an effort towards elucidating the mechanism by which the DMSO causes leakage at elevated temperatures we have studied effects of this molecule on lipid phase transitions. DMSO elevates the phase transition in direct proportion to the [DMSO]. Since the temperature at which leakage occurs is indirectly proportional to [DMSO], we concluded that leakage is not likely to be due to a phase transition. We next studied effects of DMSO on fusion between vesicles as they are heated. The results suggest that fusion can account for at least part of the leakage. We are now studying temperature-dependent effects of DMSO on order in the hydrocarbon chains of the phospholipids, using FTIR. (Supported by NSF grant DMB-85-18194.)

M-Pos130 EXCHANGE OF DMPC BETWEEN LIQUID CRYSTALLINE, GEL AND MIXED PHASE LARGE UNILAMELLAR VESICLES. William C. Wimley and Thomas E. Thompson. Department of Biochemistry, University of Virginia, Charlottesville, 22908.

The rate and extent of ^{3}[H]-DMPC exchange under equilibrium conditions were measured in DMPC liquid crystalline (LC) and gel phase large unilamellar vesicles (LUV) and also in DMPC/DSPC LC, gel and mixed phase LUV.

The exchange rates (k_{ex}) from DMPC LUV were found to be similar (28-55°C) to DPMC small sonicated unilamellar vesicles (SUV) with an Arrhenius activation energy of 23.2 ± 1.5 kcal/mol. An exchangeable fraction of ≥ 90% of the ^{3}[H]-DMPC was observed indicating that transbilayer movement (flip-flop) is fast relative to k_{ex}. This is in sharp contrast to the very slow flip-flop observed in SUV under very similar conditions.

In the binary system k_{ex} is equal to that from pure DMPC LUV above 34°C and decreases with decreasing temperature below 34°C in a way that is apparently independent of composition (25-99.9% DSPC) and phase state (LC,gel and mixed phase). At ≥ 35% DSPC and T ≥ 50°C the DMPC flip-flop appears to slow relative to k_{ex} and at 99.9% DSPC, DMPC flip-flip is immeasurably slow at all temperatures (15-55°C). At all other temperatures and compositions flip-flop is fast relative to k_{ex}.

This study demonstrates a decoupling of flip-flop and desorbtion, two processes sometimes thought to occur via high energy interfaces such as phase boundaries or spontaneous bilayer defects. The decoupling of the two processes and the lack of an effect of phase boundaries on k_{ex} implies that a simple high energy defect pathway can not be responsible for both flip-flop and desorbtion simultaneously in the system studied. (Supported by PHS NIH grant GM14628).

M-Pos131 Evaluation of Methods for Producing Liposome Particle Size Standards

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Particle size distribution has proved to be critical in determining the behavior of liposomes. As such, a great deal of effort has been applied to controlling and measuring the size and distribution of liposomes. Unfortunately, all existing methods for determination of size distribution of liposome samples have substantial limitations which must be considered when interpreting the results. Since no single method covers the complete size range required for liposomes nor satisfy the needs for every situation, it is essential to chose a method or combination of methods based on careful evaluation of the information actually needed for each situation. Consequently, homogenous size standards covering the entire range from 10 nm to 1000 nm (1 um) are essential both for comparison of results from different methods and for evaluation of the resolution and reliability of existing and new methods.

In this work an evaluation of methods for improving the homogeneity of liposome size standards up to 1000 nm will be reported. The homogeneity of samples is determined by several methods, including dynamic light scattering, light and electron microscopy. In addition, the influence of aqueous and lipid compositions which affect surface charge repulsion and bilayer fluidity will be described.

M-Pos132 LARGE RADIATION DOSES LEAVE SONICATED LIPID VESICLES INTACT. Todd P. Silverstein, Chem. Dept., Whitman Coll., Walla Walla, WA 99362 and Lester A. Braby, Radiological Physics Section, Pacific Northwest Lab - Battelle, Richland, WA 99352.

In the interest of examing the effects of radiation on very small particles we prepared sonicated phosphatidyl choline vesicles (~300 Å diameter) containing trapped internal Ca++. Ca++ efflux was followed using the Ca++ sensitive dye Arsenazo III. External Ca++ was separated from the vesicles using a Sephadex G-50 column. Baseline leakage of internal Ca++ out of the vesicles is quite slow ($\tau_{1/2}$ > 2 hrs.). Internal Ca++ was quickly and quantitatively released upon addition of A23187 to 2 uM.

Vesicle suspensions were exposed to radiation doses of up to 1 MRad (from a van de Graaf generator) with no increase of Ca++ leakage over the baseline rate on a time scale of 1-2 hours. To check if the 25 mM MOPS buffer was acting as a radioprotective radical scavenger we switched to 50 mM bicarbonate buffer with no change in results. In addition, Arsenazo III in 25 mM MOPS is completely degraded by relatively low radiation doses. In an attempt to enhance the free radical-induced lipid peroxidation reaction we added 1 mM $FeSO_4$ to catalyze a Fenton reaction, but found no effect on Ca++ release. We are presently examining several possible radiosensitizing agents to catalyze lipid peroxidation and aid in radiation-induced Ca++ release.

M-Pos133 THERMODYNAMIC CHARACTERIZATION OF LOW HYDRATION DILAUROYLPHOSPHATIDYLCHOLINE. J. M. Collins, Marquette University, Milwaukee, WI.; D. Patterson, W. Tamura-Lis and L. J. Lis, Kent State University, Kent, Ohio; and P. J. Quinn, King's College, London, U.K.

Real-time x-ray diffraction was used to examine the phase transition phenomenon for dilauroyl-phosphatidylcholine in less than excess water. In all cases a single L_C to L transition was observed at a temperature above that reported for the gel to L transition for fully hydrated DLPC. The value for T_m continuously decreased as the water content increased. This transition proceeded via a possible first order or a two phase process between acyl chain packing states in incommensurate bilayer structures. We compared this to the second order mechanism observed for the L_C to L transition in DPPC "hydrated" by water or by trehalose. Calorimetric experiments performed on similar systems produced conflicting results. Samples prepared gravimetrically indicated essentially no change in T_m until the water content increased above 25 % by weight, while those samples prepared such that the chemical potential of the system was constant showed a continuous change in T_m. Thus a "fast" temperature scan, such as that used in the real time x-ray diffraction experiments, yields similar results to those obtained calorimetrically using samples of constant chemical potential.

M-Pos134 ELECTRON DENSITY RECONSTRUCTION OF THE INVERTED HEXAGONAL (H_{II}) PHASE IN PHOSPHOLIPID-WATER MEMBRANES. D.C. Turner and S.M. Gruner,(Intro. by G.T. Reynolds) Physics Department, Princeton University, Princeton, NJ 08544.

The two dimensional electron density distribution ($\rho(r)$) of the inverted hexagonal (H_{II}) phase has been reconstructed from x-ray diffraction. A pattern recognition procedure developed by Luzzati, Mariani and Delacroix (*Macromolecular Symposium, Die Macromolecular Chemie* 15 (1988) 1-17) was used to phase the Bragg reflections by searching for a $\rho(r)$ consistent with the molecular structure of the constituents. The only previous method available for determining the internal dimensions of the H_{II} phase (Luzzati, in *Biological Membranes*, Vol. I, Academic Press, New York (1968) 71-123), requires assumptions about the structure and exact water content in the phase, non-trivial if the liquid crystal coexists with bulk water. In contrast, the electron density reconstruction can be accomplished using a single specimen with no *a priori* knowledge of the structure or the water content. Given a diffraction pattern with peaks out to (h,k) = (3,0) and a typical unit cell repeat spacing of about 70 Å, the phospholipid head group position can be determined to within 2 Å. The procedure is applied to 1,2-dioleoyl-*sn*-3-phosphoethanolamine (DOPE) to measure the structural dimensions as a function of temperature and water content. The results agree with the dimensions found by Tate (Ph.D. Thesis, Princeton University, Princeton, NJ (1987)) using the water fraction method described above. In addition, $\rho(r)$ is found to be consistent with the currently accepted model of the H_{II} phase, demonstrating that the water core is cylindrical within the measured resolution. Supported by NIH (Grant GM32614), and DOE (Grant DE-FG-02-87ER60522-A000), and an NIH Traineeship (Grant 5T32GM07312) and Garden State Graduate Fellowship to DCT.

M-Pos135 TEMPERATURE DEPENDENCE OF THE LATTICE DIMENSIONS IN THE INVERSE HEXAGONAL (H_{II}) PHASE IN MEMBRANES CONTAINING PHOSPHATIDYLETHANOLAMINE. M.W. Tate and S.M. Gruner, (Intr. by S.S. Chan) Physics Department, Princeton University, Princeton, NJ 08544.

A simple parameterization is presented for the characteristic temperature dependence of the basis vector length, d, of the fully hydrated inverse hexagonal (H_{II}) phase in phospholipid systems. X-ray diffraction shows that d falls sharply from 78.1 Å at 10°C to 62.5 Å at 90°C for 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). From the determination of the water volume fraction and the value of d, the dimensions of the lipid and water regions within the liquid crystal were calculated. This showed that the reduction in the radius of the water core, R_w, was a factor of 6 more than the reduction in the thickness of the lipid layer, $d_{H_{II}}$. The geometry of the H_{II} phase allows one to express d and R_w, both of which are nonlinear functions of temperature, in terms of $d_{H_{II}}$ and v/a, the ratio of the specific volume of the lipid molecule to the area of the lipid molecule at the water interface. The thermal coefficient of expansion of $d_{H_{II}}$ was -0.0139 Å/°C, whereas v/a was constant with T, allowing a simple parameterization of $d(T)$. Measurements on a mixture of DOPE and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) showed that $d_{H_{II}}(T)$ was similar to the DOPE system and that v/a was once again constant with temperature, although its value was different than for DOPE. It is this difference in v/a, arising from the effectively larger headgroup area of DOPC, which accounts for the larger values of d observed in the DOPE:DOPC system. Supported by NIH (Grant GM32614), DOE (Grant DE-FG02-87ER60522-A000) and a Liposome Co. Fellowship to MWT.

M-Pos136 HIGH PRESSURE X-RAY DIFFRACTION STUDIES ON THE HEXAGONAL (H_{II}) PHASE IN DOPE MEMBRANES. E. Shyamsunder, M. Novak, S.M. Gruner, Physics Department, Princeton University, Princeton, NJ 08544.

A system has been developed to obtain x-ray diffraction data under high pressure up to 1 kbar as a function of temperature up to 80°C on lipid-water samples. Experiments were performed on dioleoylphosphatidylethanolamine (DOPE) and dielaidoyl-PE (DEPE) in water. DOPE undergoes a well-known lamellar (L_α) to inverted hexagonal (H_{II}) phase transition as the temperature is raised from 2°C to 10°C at atmospheric pressure. Application of high pressure reverses the phase transition. X-ray diffraction is used to identify the phases under pressure, and shows that the increase in T_{bh}, the L_α to H_{II} phase transition temperature, with pressure p yields a value dp/dT_{bh} = 20 bar/K. Comparison with measurements on the chain melting transition temperature, T_m, in DEPE shows that the L_α-H_{II} transition temperature T_{bh} is more than two times as sensitive to pressure than T_m is. X-ray diffraction data also give the dependence of the unit cell spacings in the L_α and H_{II} phases as a function of pressure. The unit cell spacing in the H_{II} phase increases by 1 Å for every 100 bar increase in pressure. This increase is nearly 10 times greater than the increase in the unit cell spacing in the L_α phase when the same pressure is applied. A model for the L_α-H_{II} phase transition that incorporates both temperature and pressure is presented. This work was supported by NIH (Grant GM32614), DOE (Grant DE-FG02-87ER60522-A000) and ONR (Contract N00014-86-K-0396 P00001).

M-Pos137 PRESSURE-INDUCED PHASE TRANSITIONS IN BIOLOGICAL LIQUID CRYSTALS: KINETICS AND MECHANISM FROM TIME-RESOLVED X-RAY DIFFRACTION

Martin Caffrey and Andrés Mencke, Department of Chemistry, The Ohio State University, 120 West 18th Avenue, Columbus, Ohio 43210.

We describe the first use of synchrotron radiation to measure the kinetics and to decipher the mechanism of a pressure-induced phase transition in a biological liquid crystal. Time-resolved x-ray diffraction was used to continuously monitor progress of the transition and to structurally characterize coexisting phases during the interconversion following a pressure-jump perturbation.

With proper temperature control, pressure-jumps from 0 to 14MPa (0 to 2000 psi) were sufficient to effect the desired mesomorphic transformations. Here we report on a transformation between a lamellar gel (Lβ', Smectic B) and a lamellar liquid crystal (Lα, Smectic A) phase in a phospholipid/water system. Live time diffraction measurements were made in the low-angle region and focused on the (001) lamellar reflection. The transformation from Lα to Lβ' was observed to be completed in 10-11s with the application of a pressure-jump from 0 to 9.8MPa (0 to 1400psi) in 2.5-3s. The reverse transition (Lβ' to Lα) was completed in 30-32s when induced by a pressure drop from 9.8 to OMPa in 9.5-10s. Correlation of in-sample temperature with low-angle scattering as a function of time provides insights into the mechanism of the phase transformations.

In parallel with the time-resolved x-ray data collection, careful static low- and wide-angle diffraction measurements were made over the same pressure range using x-ray sensitive film. This provides an approach-to-equilibrium comparison for the kinetic data.

M-Pos138 THE INFLUENCE OF Ca^{2+} ON THE SUBGEL PHASE AND TRANSITIONS IN PHOSPHOLIPIDS.
D. Patterson, T. Mastran, W. Tamura-Lis and L. J. Lis, Kent State University, Kent, Ohio, J. M. Collins, Marquette University, Milwaukee, Wisconsin; P. J. Quinn, King's College, London, U.K.; and S. Qadri, Naval Research Laboratory, Washington, D.C.

The thermodynamic properties of dilauroylphosphatidylethanolamine, dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol in water and in low molarity $CaCl_2$ have been determined. In all cases the subgel phase was induced to assist further thermodynamic comparisons. The interaction of Ca^{2+} with DPPC is responsible for a reduction in the transition enthalpy of the subgel to gel bilayer transition with increasing $CaCl_2$ concentrations. In addition the pretransition region is broadened when 10 mM $CaCl_2$ is present. Real-time x-ray diffraction data has shown that the L_C to L_B transition proceeds via a second order thermodynamic mechanism and the broadened pre-transition phenomenon is also due to a second order transition involving a gradual loosening of the acyl chain gel state packing. The presence of very small concentrations of $CaCl_2$ eliminates the sub- and pre-transitions for DPPG bilayers and shifts the main transition temperature upward by 40° C. The Ca^{2+} interaction with DLPE appears to be biphasic. Low molarity $CaCl_2$ solutions with DLPE cause the main transition to become less thermodynamically distinct with corresponding low transition enthalpies. However, in the presence of 50 mM $CaCl_2$, DLPE bilayers undergo a distinct transition at a lower temperature than that observed for DLPE in water. From our results we infer that the degree of Ca^{2+} binding to phospholipids decreases in the sequence DPPG > DPPC > DLPE.

M-Pos139 SYNTHESIS AND THERMOTROPIC CHARACTERIZATION OF SOME NON-BILAYER FORMING DIACYL PHOSPHATIDYLCHOLINES. R.N.A.H. Lewis and R.N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta; D.C. Turner and S. M. Gruner, Department of Physics, Princeton University, Princeton, New Jersey.

The thermotropic phase behavior of phosphatidylcholines (PCs) containing acyl chains with bulky substituents near their carboxyl ends was studied by DSC, X-ray diffraction and ^{31}P-NMR spectroscopy. The presence such substituents results in a marked lowering of the gel/liquid-crystalline phase transition temperatures of these PCs and in a marked tendency for them to form highly ordered, subgel-like phases at low temperatures. However, such substituents also destabalize the lamellar liquid-crystalline phases of these PCs with respect to inverted non-bilayer phases. Our studies show that whereas inverted hexagonal (H_{II})phases are formed with the larger substituents, a decrease in the size of the substituent or an increase in its distance from the carboxyl group results in the formation of inverted cubic phases. Our findings with these 'non-bilayer forming PCs' thus suggest that with a judicious choice of appropriatedly substituted acyl chains, it should be possible to form non-bilayer phases with any of the typical 'bilayer-forming' classes of lipids. Moreover, these studies also suggest that the formation of the inverted H_{II} phases formed by these lipids may be proceeding proceed via one or more intermediates which could be inverted cubic phases or inverted micellar structures.
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M-Pos140 PROPERTIES OF MIXED ACYL PHOSPHATIDYLETHANOLAMINES. Jeffrey T. Mason and Frances A. Stephenson, The Armed Forces Institute of Pathology, Washington, D.C. 20306 (J.T.M.) and The University of Virginia, Charlottesville, Virginia 22908 (F.A.S.)

The saturated mixed acyl phosphatidylethanolamine (PE) series C(18)C(18)PE, C(18)C(16)PE, C(18)C(14)PE, C(18)C(12)PE, and C(18)C(10)PE has been prepared. The thermotropic behavior of unhydrated and hydrated aqueous preparations of these PEs has been investigated by differential scanning calorimetry and ^{31}P NMR. Unhydrated preparations of the PEs undergo crystalline to liquid-crystalline transitions (T_{m+h}), which correspond to the simultaneous hydration and acyl chain melting of poorly hydrated crystalline samples. Completely hydrated samples of the PEs undergo gel to liquid-crystalline phase transitions (T_m) when scanned immediately subsequent to cooling from temperatures above their respective T_{m+h}s. Multilamellar bilayers of C(18)C(18)PE, C(18)C(16)PE, and C(18)C(14)PE pack without significant interdigitation of their acyl chains across the bilayer center in the gel phase. C(18)C(10)PE bilayers exhibit a mixed interdigitated gel phase packing of the phospholipid acyl chains. Hydrated bilayers of C(18)C(12)PE exhibit two endothermic transitions, one at 13.9°C and a second at 36.9°C. The low-temperature transition is shown to be a mixed interdigitated gel to noninterdigitated gel transition, whereas the high-temperature transition is a noninterdigitated gel to liquid-crystalline phase transition. Supported by grant GM-33040

M-Pos141 UNUSUAL PHASE PROPERTIES OF DILAURYL PHOSPHATIDYLCHOLINE (C12PC). Michael A. Singer, Walter A. Shaw, and Leonard Finegold: Medicine, Queen's Univ. Kingston, Ont. K7L 3N6, Canada; Avanti Polar Lipids, Birmingham AL 35214; and Physics, Drexel Univ. Philadelphia PA 19104

A calorimetric scan of C12PC shows a bimodal phase pattern with a sharp endotherm located at -1.5°C (P_1) and a broader endotherm located at 5°C (P_2). Mabrey and Sturtevant (1976) initially observed P_2 and they thought it reflected an intrinsic property of the L_α state. The two peaks P_1 and P_2 can be scanned independently of one another and hence are not calorimetrically coupled. The occurrence of P_2 is also independent of scan rate over the range 0.2 to 5.0° C/min. Mixtures of C12PC and C13PC demonstrate miscibility of P_1 with the main transition of C13PC. Peak P_2 has no counterpart with C13PC. In these mixtures the temperatures of P_2 and of the combined main transition come closer together and finally overlap as the molar proportion of C13PC increases. Mixtures of C12PC and C14PC display lateral phase separation even though the acyl chains differ by only two carbons. Freeze fracture micrographs of C12PC at temperatures above P_2 show smooth faces only. Finally, purity studies of C12PC (TLC, P-31 NMR, HPLC, GC) indicate the sample to be better than 99% pure. We propose that P_2 represents a new and as yet to be defined transition occurring between the gel and L_α state.

(Support: MRC, Drexel Faculty Leave Plan)

M-Pos142 CALORIMETRIC STUDIES OF A SERIES OF CHIRAL AND RACEMIC GLYCOSYL DIALKYL GLYCEROLS. D. A. Mannock, R.N.A.H. Lewis and R. N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton Alberta, Canada; M. Akiyama, Department of Physics, Sapporo Medical College, Sapporo Japan.

Glycosyl dialkylglycerols are membrane constituents of many Archaebacteria which live in extreme environments. Although these compounds have been used as analogues of their more widespread diacyl counterparts, the studies on these compounds have not been very detailed and have concentrated on the 1,2-sn-enantiomers, which are not naturally occurring. We have synthesized a series of glycosyl dialkyl glycerols in which the hydrocarbon chain lengths and chirality of the glycerol backbone have been systematically varied, and studied their thermotropic phase behavior by DSC and X-ray diffraction. The phase behavior of both the chiral compounds studied are fairly complex phenomena which involve gel-phase polymorphism, chain-melting events and interconversions between bilayer and non-bilayer structures. With the racemic compounds, however, gel-phase polymorphism is absent and only those thermotropic events attributable to chain-melting events and interconversions between lamellar and non-lamellar phases are observed. Both the racemic and chiral compounds exhibit a similar pattern of bilayer/non-bilayer interconversion which is strongly dependent upon the length of the hydrocarbon chain. The shorter-chain compounds exhibit discrete lamellar/cubic and cubic/inverted hexagonal transitions whereas the longer chain compounds only exhibit lamellar/inverted hexagonal transitions. Our results indicate that despite general similarities in physical properties of these glucosyl dialkylglycerols and their diacyl counterparts, there are significant behavioral differences particularly with respect to the gel-phase polymorphism. This suggests that the structure of the interfacial region of a lipid molecule is a significant determinant of its physical properties. (Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.)

M-Pos143 ***A EUTECTIC PHASE DIAGRAM FOR BINARY MIXTURES OF SYMMETRIC AND ASYMMETRIC PHOSPHATIDYLCHOLINES WITH IDENTICAL MOLECULAR WEIGHT: A CALORIMETRIC STUDY.*** ***R. B. Sisk Dept. of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.***

The thermotropic behavior of aqueous dispersions of C(22):C(12)PC and C(17):C(17)PC mixtures at varying molar ratios was investigated by high-resolution differential scanning calorimetry. C(22):C(12)PC is a highly asymmetric lipid equal in molecular weight to the symmetric lipid C(17):C(17)PC. Calorimetric results indicate that these two lipids are completely miscible in the liquid-crystalline phase and are generally immiscible in the gel phase. A temperature-composition phase diagram for binary mixtures of these lipids can be constructed based on the onset and completion temperatures of the transition curves. It exhibits the characteristic shape indicative of a eutectic system. The observed eutectic behavior is consistent with the similar packing of the component lipids at $T > Tm$ and with their dissimilar packing at $T < Tm$. Eutectic behavior may have important physiological significance in the regulation of structure and dynamics of two-dimensional spatial domains of the lipid matrix in biological membranes. Supported by USPHS Grant GM-17452.

M-Pos144 TIME-RESOLVED FLUORESCENCE STUDIES OF "ZINC FINGER" PROTEIN TRANSCRIPTION FACTOR IIIA (TFIIIA). M. Han[1], J. Knutson[2], S. Kim[*3], M. Fisher[1], F. Cyran[1], and A. Ginsburg[1]. [1]LB and [2]LTD, NHLBI, NIH, Bethesda, Maryland 20892; and [*3]Carnegie Institute, Baltimore, MD 21210.

TFIIIA is stored in large quantities as 7S-Particles (a complex of TFIIIA and 5SRNA) in immature oocytes of *Xenopus laevis*. The TFIIIA (M_r 38,000) consists of a N-terminal domain that binds to the internal control region of the 5SRNA gene and a C-terminal domain that is essential for transcription. The amino terminal segment contains 9 "zinc fingers", each proposed to fold around a central Zn^{2+} ion. Included in this domain are two tryptophanyl and many tyrosinyl residues; these serve as intrinsic probes. Time-resolved fluorescence decay measurements on the native protein were best fit by three lifetime components of 1.0 ns (30%), 3.7 ns (65%), and 7.1 ns (5%) when excited at 295 nm (340 nm emission). Using global analysis of decay curves at many emission wavelengths, these terms are linked to distinct decay-associated spectra. The short-lived spectrum was found to be considerably blue-shifted from the others. Further, the Trp residues are largely immobilized (as seen via emission anisotropy decay); the dominant 25 ns rotational correlation time is a typical value for a protein of this size. Mercurial reagents promote the rapid release of Zn^{2+} from TFIIIA, as monitored with the metallochromic indicator 4-(2-pyridylazo)resorcinol. After Zn^{2+} release, TFIIIA emission is composed of two red-shifted spectral components with lifetimes of 1.8 ns (63%) and 5.2 ns (37%). Thus, the emission decays of Trp residues in TFIIIA reflect conformational changes that occur upon Zn^{2+} removal. Combined steady-state and kinetic spectroscopic techniques are providing additional information on nucleic acid-protein and protein-protein interactions with the TFIIIA system.

M-Pos145 The microenvironment of sickle hemoglobin contact sites as seen by spin-probe-spin-label techniques

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The spin-labeled tryptophan was used as a structural probe of hemoglobin contact sites. The ESR spectral data indicated that the probe exhibits weak binding to hemoglobin with a dissociation constant of $3.2.10^{-5}$ and 4.0 mol bound per hemoglobin tetramer. The spectrum suggested that the bound tryptophan was 'partially immobilized' with a correlation time reflecting the environment of the tryptophan binding site of 8.2 ns. The topology of the contact sites was investigated by using dual spin-label methodology in which spin-labeled tryptophan and (^2H,^{15}N) substituted and deuterated maleimide spin label [^2H-^{15}N]ISL covalently-bound to Cys-93 residue were used. The ESR spectral data suggested that the tryptophan binding sites were located within 8-10 Å of the nitroxide free radical of spin-labeled hemoglobin. The environment of the contact sites is discussed.

M-Pos146 Time Resolved and Steady State Fluorescence Studies of the Dansyltyrosine derivatives of Porcine Pancreatic Colipase. J. C. McIntyre, F. Schroeder, and W. D. Behnke, University of Cincinnati College of Medicine, Dept. of Biochemistry and The College of Pharmacy, University of Cincinnati.

Through a series of nitration, reduction, acylation and dansylation reactions followed by column chromatography, we have obtained two derivatives of pancreatic colipase; 1DNSPC-dansyltyrosine 55, and 2DNSPC-N-acyltyrosine 55-dansyltyrosine 59. The 1DNSPC derivative has 200% the activity of the unmodified protein while the 2DNSPC is 80% active. The steady state emission spectrum of 1DNSPC has an em-max of 550nm (surface exposed). Upon titration with Taurodeoxycholate (a model system for bile salt emulsified fat droplets) the spectrum showed a 4 fold increase in Quantum yield and a 70nm blue shift. The 2DNSPC derivative has an emission maxima of 520nm which does not change upon TDOC titration but the quantum yield does increase 2.3 fold. The acrylamide quenching constants Ksv and Kq obtained in the presence and absence of CMC levels of TDOC confirmed that DNStyr55 is a surface residue that becomes buried upon the addition of TDOC. Quenching also showed that DNStyr59 is more buried compared to DNStyr55 and the addition of TDOC does not hinder quenching but increases its efficiency. The lifetime of 1DNSPC has two components t1=11.761ns(74%) and t2=1.323ns (26%), 2DNSPC also fits a two component fit, t1=9.920 (68%) and t2=1.542ns (32%). Upon titration with TDOC, t1 of 1DNSPC increased 45% while t1 of 2DNSPC showed no specific changes. The differential polarization (P) value for 1DNSPC is P=.052 which increases to P=.110 in the presence of 12.5mM TDOC while P=.121 for 2DNSPC and P=.100 with 12.5mM TDOC. To assess the influence of increased size of the PC-TDOC complex on P, we measured the differential polarization of a dansyl derivative of colipase that shows no specific fluorophore micelle interaction. In this case P only shows very small increase upon TDOC binding.

M-Pos147 RESONANCE ENERGY TRANSFER BETWEEN THE ACTIVE SITES OF RABBIT MUSCLE CREATINE KINASE.
Steven H. Grossman, Chemistry Department, University of South Florida, Tampa, FL 33620.
Resonance energy transfer between the reactive thiols of rabbit muscle creatine kinase (CK-MM) was evaluated. The reactive thiols are located at the active site, one occurring on each subunit of the dimeric protein which is known to be a constituent of the M-line structure of the myofibril. Donor/acceptor labelled proteins were prepared by subunit hybridization or sequential reaction with fluorophores exhibiting biphasic kinetics. Disproportionation of singly labelled dimers (to unlabelled and doubly labelled dimers) was not observed using the brain isozyme of CK to trap dissociated dye-conjugated or unlabelled muscle type subunit of CK. Transfer efficiency was evaluated from donor quenching using steady-state and phase/modulation lifetime measurements and determination of sensitized acceptor emission. Six different donor/acceptor combinations displayed transfer efficiencies from 4 to 19%, indicating that the reactive thiols are well-separated. Measurement of quantum yields and estimates of the range of orientation factor provide a limited distance of 48.6 to 60.4 A between the active sites. Phase resolved anisotropy measurements yield a rotational correlation time of approximately 40 to 50 nsec, and faster motions consistent with probe rotation or segmental flexibility. The site-site distance of CK-MM is longer than the hybrid, myocardial specific isozyme, CK-MB [Grossman, S. H. (1983) Biochemistry 22 5369-5375] suggesting potential differences in subunit arrangement which could account for isozyme specific compartmentation. Position of the active sites also suggests that localized conformational changes accompanying catalysis would have negligible influence on associated myosin rod structure.
(Supported in part by NIH grant NS-23396).

M-Pos148 TIME RESOLVED FLUORESCENCE AND ANISOTROPY OF COMPLEXED INDOLE DERIVATIVES

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Radiative and non-radiative decay mechanisms compete to deactivate the excited state of indole derivatives. A number of quenching mechanisms have been suggested to exist, with the efficiency of quenching shown to depend upon the solvent and the nature of the solvent-solute interactions. These decay mechanisms are generally measured in solution phase, low temperature glasses, or high viscosity glycerol solutions. While much of the work has been aimed at describing the excited state processes to which indole derivatives are susceptible, static charge perturbations of ground and excited state molecular properties may effect many of the decay mechanisms. We have studied radiative and non-radiative decay mechanisms of complexed indole derivatives at room temperatures to assess decay mechanisms that may be pertinent to constrained tryptophan within protein molecules. Methyl and carboxylic derivatives of indole were complexed within β-cyclodextrin, and the fluorescence and quenching processes studied with time resolved fluorescence, anisotropy, circular dichroism and magnetic circular dichroism measurements. We believe these systems can provide reasonable approximations to the effects of static charge perturbations to the ground and excited state indole chromophore. Specifically, we have observed that carboxylic groups substituted at certain positions of the indole ring effect the nature of S_0-S_1 electronic transitions as shown by magnetic circular dichroism studies, and that these substitutions have dramatic effect on the fluorescence lifetime. We intend to relate different substitutions on the indole ring to non-radiative decay processes, and to differentiate intramolecular from solvent induced quenching processes.

M-Pos149 DISTRIBUTION OF DISTANCES IN THE RANDOM COIL AND α-HELICAL STATES OF MELITTIN FROM FREQUENCY-DOMAIN FLUOROMETRY. Joseph R. Lakowicz, Ignacy Gryczynski, Gabor Laczko, Wieslaw Wiczk, and Henryk Szmacinski, University of Maryland, Department of Biochemistry, Baltimore, MD, Franklin G. Prendergast, Mayo Foundation, Rochester, MN, and Michael L. Johnson, University of Virginia, Department of Pharmacology, Charlottesville, VA.

We used fluorescence energy transfer to examine the effects of solvent composition and complexation on the distribution of distances between the single tryptophan residue of melittin (residue 19) to the N-terminal α-amino group, which was labeled with a dansyl residue. The tryptophan intensity decays, with and without the dansyl acceptor, were measured by the frequency-domain method. The data were analyzed by a least-squares algorithm which accounts for correlation between the parameters. A wide distribution of tryptophan-to-dansyl distances was found for the random coil state, with a Gaussian half-width of 25 A. Increasing concentrations of methanol, which were shown to induce an α-helical conformation, resulted in a progressive decrease in the width of the distribution, reaching a limiting half-width of 3 A with 80% methanol. Narrow distance distributions were also found for melittin complexed with calmodulin, 8.2 A, or with POPC vesicles, 4.9 A. A somewhat wider distribution was found for the melittin complex with TnC, 12.8 A, suggesting the presence of heterogeneity in the mode of binding between melittin and TnC. These results demonstrate the usefulness of frequency-domain measurements of non-radiative energy transfer for resolution of conformational distributions of proteins.

M-Pos150 **FLUORESCENCE STUDIES WITH TWO SITE-DIRECTED MUTANTS OF STAPHYLOCOCCAL NUCLEASE A.** Maurice R. Eftink[1] and Camillo A. Ghiron[2], [1]Dept. of Chemistry, Univ. of Mississippi, University, MS 38677, and [2]Dept. of Biochemistry, University of Missouri, Columbia, MO 65211.

Two mutants of nuclease A, PG117 and PA56, prepared by site-directed mutagenesis by Evans etal ((1987) Nature 329, 266), have been studied using fluorescence methods to probe the state of the single trp-140 in these proteins. The mutant PG117 has a gly in place of pro-117. Fluorescence lifetime measurements were made with PG117 and wild type nuclease, as a function of temperature (T), to test the hypothesis that two conformations exist in the latter due to the slow cis→trans isomerism of pro-117 and that this is the molecular basis for the bi-exponential decay of the trp emission of the wild type. Lifetime data were found to be similar for the wild type and PG117, discounting this isomerism as a source of the fluorescence heterogeneity.

PA56, which has ala in place of pro-56, was found to have a very low thermal transition temperature, T_m, of 30°C and a ΔH_m^o for unfolding of 48.5 kcal/mole at pH 7. These values are very small in comparison to the wild type (T_m = 53°C, ΔH_m^o = 86 kcal/mole). PA56 is denatured by a very low concentration of guanidine HCl (50% denatured at 0.15 M). Both thermal and guanidine denaturation studies indicate that the ΔG^o for unfolding of PA56 is only 1.7 ± 0.1 kcal/mole at 20°C. This low stability of PA56 is sensed by acrylamide quenching and fluorescence lifetime measurements. The addition of acrylamide, between 20-30°C, actually potentiates unfolding and results in enhanced quenching. Lifetime measurements, as a function of T, show the decay of trp-140 to become more non-exponential near T_m. A global analysis of lifetime data will be presented in terms of a bimodal distribution of decay times. A two state unfolding model accounts for all of the fluorescence data on PA56. Research supported by NSF DMB 88-06113.

M-Pos151 ELECTRIC BIREFRINGENCE STUDIES OF THE TROPONIN-TROPOMYOSIN COMPLEX. Charles A. Swenson and Nancy C. Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

The association of troponin with non-polymerizable tropomyosin has been studied using electric birefringence measurements on the solutions. The measurements were made as a function of concentration, temperature, ionic strength, pulse length and electric field strength in the presence and absence of calcium ion. The zero field birefringence decay was resolved into two rotational relaxation times; one characteristic of tropomyosin and one characteristic of the troponin-tropomyosin complex which is several fold larger. We will also present some information on the electro-optical properties of the complex.

M-Pos152 **DISTRIBUTION OF DISTANCES IN SKELETAL TROPONIN C AND ITS COMPLEX WITH TROPONIN I FROM FREQUENCY-DOMAIN MEASUREMENTS OF FLUORESCENCE ENERGY TRANSFER.** Herbert C. Cheung and C.K. Wang, University of Alabama, Department of Biochemistry, Birmingham, AL, Ignacy Gryczynski, Wieslaw Wiczk, Gabor Laczko, Robert F. Steiner, and Joseph R. Lakowicz, University of Maryland, Department of Biochemistry, Baltimore, MD, and Michael L. Johnson, University of Virginia, Department of Pharmacology, Charlottesville, VA.

We used frequency-domain fluorescence energy transfer to determine the distribution of donor-to-acceptor distances in skeletal troponin C (TnC) and its complex with troponin I (TnI). Dansylaziridine (DNZ) attached to Met-25 was used as the energy donor (D) and 5-(iodoacetamido) eosin (IAA) linked to Cys-98 as the acceptor (A). The frequency-response of the donor fluorescence, in the absence and presence of acceptor, was used to recover the distribution of D to A distances. At pH = 7.5 the D-A distribution is characterized by an average distance of 22.4 Å and a half width 13.3 Å. In the presence of Ca^{2+} the half-width appeared to decrease slightly to 10.9 Å. At pH = 5.2 we observed significant changes in the D-A distribution; the average distance increased to 31.8 Å and the half width decreased to 7 Å. At acidic pH, TnC appears to become more extended and/or rigid. This fact was also observed in the anisotropy decay as an increase of the correlation time from 12 ns to 19 ns. At this time we cannot exclude dimerization of TnC at pH 5.2, but believe dimerization is unlikely at these concentrations. Unfolding in guanidine hydrochloride resulted in a very broad D-A distribution, 30-40 Å in width.

M-Pos153 FLUORESCENT-NUCLEOTIDE PROBES OF STRUCTURAL CHANGES TO p21ras DURING GTP HYDROLYSIS. Susan E. Neal, Jackie L. Hunter, John F. Eccleston and Martin R. Webb, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. (Intr. by David R. Trentham)
p21, the protein expressed by the proto-oncogene ras probably has a regulatory role in cell signalling mediated by its GTPase activity. We are investigating how this activity may be related to signal activation in the normal protein and mutants expressed by ras oncogenes. We have measured most rate constants for the minimal GTPase mechanism using single turnover conditions. Pi release and rebinding have been probed by Pi-water oxygen exchange, and Pi release is probably at least 10-fold faster than the hydrolysis step. These measurements show no significant kinetic differences between GDP and GTP for binding and release and no direct evidence for protein conformation changes, which are likely to be important in the activation cycle. We are using fluorescent nucleotide analogues to probe structural aspects of the nucleotide-protein interactions. Modifications to the ribose ring of the nucleotide have little affect on the binding to p21. 2'(3')-(N-methyl)anthraniloyl-GTP (Mant-GTP) shows a 2-fold fluorescence enhancement on binding to p21, as does Mant-GDP. Polarization is about .30 for both Mant-GDP and Mant-GTP complexes. Acrylamide quenching of the Mant-GTP complex is more efficient than for the Mant-GDP complex, presumably due to a conformation difference between these complexes. (Supported by the MRC, U.K.)

M-Pos154 RAMAN AND INFRARED SPECTROSCOPIC STUDIES OF THE MIXTURE OF POLY-L-LYSINE·HBr AND POLY-D-LYDINE·HBr. Vaman M. Naik (intr. by J. Bandekar), Department of Natural Sciences, University of Michigan-Dearborn, Dearborn, MI 48128

We have characterized the conformations of poly-L-lysine HBr·(PLL) and poly-D-lysine HBr·(PDL) and the precipitate formed by mixing equal volumes of aqueous alkaline solutions of PLL and PDL. PLL (mean chain length, n=481) and PDL (n=437) adopt each an α-helical conformation in aqueous alkaline solutions (0.03 monomerl/L, pH=11). But the dried precipitate from the racemic mixture shows infrared (ir) and Raman (R) amide I and II bands that are characteristic of the antiparallel β-sheet structure: 1625 cm^{-1} (ir,s), 1669 cm^{-1} (R,s), and 1694 cm^{-1} (ir,w), 1530 cm^{-1} (ir,s) and 1558 cm^{-1} (R,w) (w=weak, s=strong). If the mixture of L- and D- chains is not racemic, then the β-sheet conformation is not obtained. This shows that the L- and D- chains alternate in the sheet to form inter-chain hydrogen bonding. These results and Raman and ir spectra of the α-helical conformations will be presented.

The author wishes to thank Prof. S. Krimm for providing the use of his spectroscopy laboratory and Dr. J. Bandekar for useful discussions.

M-Pos155 SPECTROSCOPIC INVESTIGATION OF THE INTERACTION OF TRIFLUOPERAZINE WITH BOVINE BRAIN S100a PROTEIN. P. L. Pingerelli, B.A. Wachocki, and H. Mizukami. Division of Regulatory Biology and Biophysics, Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202.

S100a is a calcium binding protein belonging to a family of proteins termed S100. Presently, the physiological significance and whether each member of the S100 family has multiple or specific functions is not clear. However, it is known that S100 proteins bind to many calmodulin target molecules in a calcium dependent manner. These observations suggest that S100 proteins may function in vivo as calcium-modulated effector proteins. In this report, the calmodulin antagonist, trifluoperazine (TFP), was utilized as a hydrophobic probe to study the Ca^{2+}-dependent binding properties of S100a.

Proton and fluorine NMR were used to study the interaction of TFP with S100a. Upon addition of protein, the fluorine resonance of TFP shifted downfield 0.36 ppm and was saturated at drug: protein ratio of 2:1. The exchange rate for Ca^{2+}-bound S100a was estimated to be 3×10^2 s^{-1} and is slower than the 9×10^2 s^{-1} TFP exchange rate we have previously reported for the S100b isoform in the presence of excess Ca^{2+}. Proton NMR spectra of S100a/TFP solutions suggest phenlyalanine residues are involved in binding of TFP.

The interaction of TFP with isoforms from the S100 family has been a useful model probe to identify structural changes. Our observations suggest that Ca^{2+}-dependent binding sites of TFP on S100a are structurally different from Ca^{2+}-dependent sites on S100b protein and indicates each isoform may have different functions. (supported in part by grants from the Graduate School, WSU.)

M-Pos156 FLUORESCENCE SPECTROSCOPIC STUDIES OF PLANT CALMODULIN AND ITS PEPTIDE COMPLEXES.
Gautam Sanyal*, Faith M. Thompson*, Henry Zot+ and David Puett+. *Department of Chemistry, Hamilton College, Clinton, NY 13323 and +Department of Biochemistry, University of Miami School of Medicine, Miami, FL 33136. The effect of ligand binding to plant CaM (PCaM) on the environment of its single intrinsic fluorophore, tyrosine-138 (Y-138), was studied. The accessibility of Y-138 to dynamic fluorescence quenchers iodide and succinimide was Ca^{2+}-dependent. Accessibility to iodide was significantly decreased upon transition from a Ca^{2+}-free to a Ca^{2+}-bound form of PCaM. The binding of the non-fluorescent peptide mastoparan to PCaM, in the presence of Ca^{2+}, increased the quantum yield (ϕ) of Y-138; concomitantly Y-138 became more accessible to both iodide and succinimide. Binding of amphiphilic peptides to PCaM occurred with high affinity (Ca^{2+}-dependent), as indicated by PCaM-induced changes in the fluorescence anisotropy (r) and spectrum of the single tryptophan (W) of each peptide. The dependence of spectral blueshifts and acrylamide-accessibility of W on its position in the sequence were consistent with an apolar-apolar interaction between a PCaM helix and the peptide helix. Our observation here is similar to earlier findings for mammalian CaM. (McDowell et.al., Biochemistry 24, 2979, 1985, O'Neil et.al., Science 236, 1454, 1987.) However, the ϕ and lifetime (τ) values for the PCaM-bound peptides (τ was measured by multifrequency phase fluorometry) did not show any correlation with the position of W in the putative peptide helix. These data suggest that local electrostatic interactions with residues on the interacting PCaM surface are important in determining ϕ and τ once the protein-peptide interface has formed. Similar findings have been recently made for peptides bound to mammalian CaM (Sanyal and Prendergast, Fluorescent Biomolecules, Plenum, pp. 401-405, in press). (Supported by NIH and PRF Grants)

M-Pos157 **ESTIMATION OF THE SECONDARY STRUCTURE OF PROTEINS IN AQUEOUS SOLUTIONS FROM THEIR FTIR SPECTRA**

Françoise DOUSSEAU, Marie THERRIEN, Michel PEZOLET
Département de chimie, Université Laval, Québec, Québec, G1K7P4.

A simple method for the quantitative determimation of the secondary structure of proteins in aqueous solutions by infrared spectroscopy has been developped. The strong band at 1645 cm^{-1}, due to the water bending mode, which interferes with the proteins amide I and II bands was first subtracted quatitatively from the transmission spectra of the proteins in solution using a least-squares algorithm. The internal intensity standard for the subtraction was the association band of water at 2130 cm^{-1}. Spectra of several proteins of known secondary structures were recorded and used to calculate spectra of pure classes of conformation such as α-helical, β-sheet and undefined structures. To test the accuracy of the method, a linear combination of these reference spectra were then fitted to the spectra of proteins with known structures in order to obtain estimates of their secondary structure contents. The results show that the correlation coefficient between the X-ray and FTIR data is close to 0.9 for the α-helical and β-sheet structures but slightly lower for the undefined structure.

M-Pos158 SIMULTANEOUS DETERMINATION OF INTRAMOLECULAR DISTANCE DISTRIBUTIONS AND CONFORMATIONAL DYNAMICS BY GLOBAL ANALYSIS OF FLUORESCENCE ENERGY TRANSFER MEASUREMENTS.
Joseph M. Beechem and Elisha Haas, Lab. for Fluorescence Dynamics, Dept. of Physics, Univ. of Illinois-UC, Urbana, IL 61801 and Dept. of Chem. Physics, Weizmann Inst. of Science, Rehovet, Israel.

Fluorescence energy transfer is widely used for determination of intramolecular distances in macromolecules. The time dependence of the rate of energy transfer is a function of the donor-acceptor distance distribution and fluctuations between the various conformations which may occur during the lifetime of the excited-state. Previous attempts to recover both distance distributions and segmental diffusion from time resolved experiments were unsuccessful due to the extreme cross-correlations between fitting parameters. A method has been developed, based on a global analysis of both donor and acceptor fluorescence decay curves, which overcomes this this extreme correlation and allows the parameters of the equilibrium distance distribution(s) and intramolecular diffusion constants to be recovered with high statistical significance and accuracy. Simulation studies of typical intramolecular energy transfer experiments reveal that both static and dynamic conformation information can thus be obtained at a single temperature and viscosity. Since both distance distributions and translational diffusion can now be simultaneously recovered, the effects of rotational diffusion on the energy-transfer time-dependent decays is now being examined. The theory required to incorporate the orientational dependence (together with distance distribution and translational diffusion) is developed. With this theory, the possibility of analyzing the time-resolved anisotropy of energy-transfer measurements is discussed.
JMB is a L.P.Markey scholar in Biomed. Science; EH supported by NIH GM 39372-01.
JMB thanks Dr. B. Wieb Van Der Meer for helpful discussions.

M-Pos159 **FLUORESCENCE STUDIES ON MYELIN BASIC PROTEIN: SALT AND pH-CONTROL OF PROTEIN CONFORMATION**, by Mark W. Nowak and Harvey Alan Berman, Department of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, New York 14260.

Myelin basic protein (MBP) exists as a class of cytoplasmic proteins characterized by high isoelectric points (>11.5). Since these proteins are highly basic, the pH and ionic nature of the cytoplasmic environment might be expected to influence MBP conformation and function. To examine the influence of pH and ionic strength on MBP conformation, we monitored the intrinsic steady-state fluorescence intensity, trp-115, of the 18.5 kD form derived from bovine white matter. At pH 7.4, titration of bovine MBP with NaCl, KCl, $MgCl_2$, and $CaCl_2$ caused measurable, concentration-dependent increases (19+/-1% at 1 M NaCl) in tryptophanyl fluorescence. The effects seen at pH 4.5 were measurably less than at the higher pH. Bimolecular constants for quenching of tryptophanyl fluorescence by acrylamide were uniformly high (> 10^9 M^{-1}-sec^{-1}) suggesting that the tryptophanyl residue of MBP was highly accessible to the bulk aqueous medium. These results reveal that MBP conformation is highly mobile and dependent on pH and ionic strength of the medium. This conclusion will be discussed with respect to conformation, cytoplasmic localization, and a functional role for MBP in mediating myelin compaction.

M-Pos160 THE ROLE OF CHARGE DIFFERENCES ON MYELIN BASIC PROTEIN ISOFORM STRUCTURES. C. Caamano, J. Azcura, O.Z. Sellinger and R. Zand, Biophysics Research Division, Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

An attractive experimental paradigm that would permit the assessment of the role of charge differences on the structural organization of protein charge isoforms, is to utilize the charge isoforms as substrates in an enzyme catalyzed kinetic study. Differences in the various kinetic parameters can be interpreted as reflecting differences in the structure of this molecular family.

The charge isoforms (C1 through C5) of bovine myelin basic protein, were used as substrates for the rat brain enzyme protein carboxylmethyltransferase (PMII). Initial velocity plots as a function of the MBP-isoform concentration showed significant differences ($P<0.05$) among the assayed isoforms except for isoforms C2 and C4. Under the conditions of our experiment all the curves exhibited a consistent sigmoidicity. The kinetic data were best fitted by a model previously described for the enzyme D-β-hydroxybutyrate dehydrogenase, in which two independent sites must be randomly occupied before any catalytic activity can occur. This mechanism is substantially different from that proposed by other investigators for similar PM II enzymes and other substrates. The differences in the rates of isoform carboxylmethylation are largely accounted for by the different apparent dissociation constants Ks, and is explained on the basis of inherent structural differences among the charge isoforms.

This research was supported in part by U.S. Public Health Service Grant NS23474 to R. Zand and AG06071 to O.Z. Sellinger.

M-Pos161 FACTORS IN THE FOLDING OF BACTERIORHODOPSIN. Authors: T.W. Kahn, B.D. Adair, H. Sigrist, and D.M. Engelman, Dept. of Molecular Biophysics and Biochemistry, Yale University, *Universitat Bern, Switzerland.

Factors involved in the folding of bacteriorhodopsin (BR) may include the polypeptide links between helices and packing effects within the lipid environment. Studies of these two factors will be reported.

It has previously been shown that BR cleaved between the second and third helix will refold to a structure that can be induced to form the purple membrane lattice (1). This lattice can be reformed even when the two fragments are denatured and reconstituted. We have now studied a similar cleavage produced by staphylococcal V8 protease between the fifth and sixth helices. Again, an x-ray diffraction pattern was obtained indicating the formation of a purple membrane lattice having molecules similar in structure to native BR. Thus, it appears that neither of these two links between helices is essential for the reformation of the correct BR structure from reconstituted fragments.

To probe the role of packing effects, we have been using small molecules added to the lipid bilayer to fill the packing voids that would otherwise be created at the protein-lipid interface by projecting sidechains. Our preliminary results suggest that the reassociation of the helices in a lipid bilayer under conditions of reconstitution is reversibly blocked by the presence of such small molecules as 2,2 dimethylbutane, 2,3 dimethylbutane, etc. Several control experiments are now in progress, and the results will be discussed at the meeting.

1. Popot, J-L, Trewhella, J., and Engelman, D.M. (1986) EMBO J. 5:3039

M-Pos162 PERTURBATION OF THE ABSORPTION SPECTRUM AND STRUCTURE OF BACTERIORHODOPSIN IN PHOSPHATIDYLCHOLINE VESICLES BY ACYL CHAIN AND PH VARIATION. Barbara A. Lewis, Department of Chemistry, University of Wisconsin, Madison WI 53706.

Bacteriorhodopsin (BR) is an ideal model membrane protein. By performing solid state NMR and optical experiments on reconstituted vesicles made with delipidated BR and a range of lipids, we are exploring the interactions of BR with its lipid and ionic milieu. The goal is to elucidate the roles of hydrophobic and electrostatic forces in determining the structure and function of this model transmembrane protein.

When delipidated BR is reconstituted into phosphatidylcholine (PC) vesicles, a blue-shifted species ($\lambda_{max} \simeq 490$ nm) appears at alkaline pH. The apparent pK_a for this transition in BR-PC vesicles made with either short-chain saturated or long-chain cis-monounsaturated PCs are lower than for the longer-chain saturated PCs. This suggests that lipid bilayer thickness is one, but not the only, lipid-related determinant of λ_{max}. Solid-state ^{13}C NMR studies of peptide-labeled BR in these vesicles are in progress. Using the approach previously described (Lewis, et.al. (1985) Biochemistry 24: 4671), lipid and pH effects on the average peptide bond orientation (i.e., helix tilt angles) are determined.

Supported by N.I.H. GM38532.

M-Pos163 MODEL FOR SHORT-CHAIN PC MICELLES BASED ON SANS DATA QUANTITATIVELY ACCOUNTS FOR PHOSPHOLIPASE C (*BACILLUS CEREUS*) "SUBSTRATE INHIBITION", Mary F. Roberts, Tsang-Ling Lin*, and Sow-Hsin Chen*, Department of Chemistry, Boston College, and *Department of Nuclear Engineering, M.I. T., Cambridge, MA 02139

SANS data for a series of symmetric and asymmetric short-chain lecithin micelles uniquely provides both the structure and thermodynamic parameters of these micellar systems. The growth of these spherocylinder micelles in the longitudinal direction with increasing PC concentration is due to the free energy of insertion of a monomer in the straight section (rod) being lower than that in the end caps. This free energy difference, extracted from the SANS analysis, is attributed to the smaller surface area of the hydrocarbon core in contact with water for a monomer in the rod compared to that in the end caps. Short-chain PC micelles are excellent substrates for water-soluble phospholipase C from *Bacillus cereus*. That enzyme shows an unusual kinetic pattern of apparent "substrate inhibition" at high diheptanoyl-PC concentrations. Rather than proposing a second inhibitory PC site on the enzyme, we can rationalize this decrease in activity by proposing that the enzyme sees two distinct PC substrates, i.e., it has a different V_{max} and K_m for PC in the end cap versus the rod sections. The thermodynamic model for short-chain PC micelles allows us to estimate the amount of end cap vs. rod PC molecules at any total PC concentration. Using this information we can fit the kinetic data for diheptanoyl-PC with $V_{max}^{ec} = 2650$ umol $min^{-1}mg^{-1}$, $K_m^{ec} = 0.02$ mM, $K_m^{rod} = 0.13$-0.15 mM, $V_{max}^{rod} = 75$-300 umol $min^{-1}mg^{-1}$. Extension of this treatment to other micelles and bilayers indicates that for phospholipase C the area/head group of the phospholipid is critical, primarily by affecting "productive" substrate binding.

M-Pos164 PROTEOLYSIS OF MEMBRANE-BOUND 3-HYDROXYBUTYRATE DEHYDROGENASE (BDH). Thomas M. Duncan, J. Oliver McIntyre, Faryab Lohrasbi and Sidney Fleischer. (Intr. by Linda J. Van Eldik). Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

BDH, a mitochondrial membrane-bound enzyme, has a specific requirement of phosphatidylcholine (PC) for function. BDH can be reconstituted unidirectionally with preformed phospholipid vesicles or natural membranes, but only PC-containing membranes activate the enzyme. Previous studies showed that apo-BDH (devoid of lipid) and BDH reconstituted with membranes lacking PC are rapidly cleaved and/or inactivated by a number of endo- and exo-peptideases. By contrast, native BDH (in sub-mitochondrial vesicles, SMV) or BDH reconstituted with PC-containing vesicles are protected from cleavage and/or inactivation. With chymotrypsin (ChT), cleavage of BDH reconstituted with PC-containing vesicles is unique in that BDH (~ 31 Kd) is cleaved rapidly, yielding a fragment of ~ 25 Kd (F~25), while loss of BDH activity occurs more slowly. Now, using BDH-specific polyclonal antibodies for detection, we have reinvestigated the cleavage of BDH by ChT. We find that the cleavage pattern of BDH is similar whether it is cleaved in SMV or after reconstitution with PC-containing vesicles or rat liver microsomal membranes. BDH from two different species (bovine heart and rat liver) show similar sensitivities to ChT in SMV and both yield a stable F~25. F~25, which appears to retain partial activity, is shown to remain membrane-bound. Active site ligands, NAD and 2-methylmalonate, provide added protection of both BDH and F~25 against further cleavage by ChT. We conclude that: 1) BDH has a similar conformation in SMV and reconstituted with PC-containing membranes; 2) in the presence of PC, a fragment (F~25) of BDH is resistant to cleavage by ChT; and 3) F~25 remains membrane-bound and appears to retain partial activity.

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M-Pos165 EFFECT OF LUNG SURFACTANT PROTEIN C (SP-C) ON THE ORDERING OF PHOSPHOLIPID BILAYERS Barry W. Elledge and Jeffrey A. Whitsett (Intr. by Kevin Schlueter) Pediatrics Dept., University of Cincinnati College of Medicine and Children's Hospital Research Foundation, Cincinnati, OH 45267

The hydrophobic apoproteins found as minor components of lung surfactant are essential for conferring proper biophysical activity on their associated phospholipids (PL). The valine-rich 36 amino acid peptide SP-C converts poorly active PL mixtures to highly active surfactants. The alterations in PL bilayer physical properties which are induced by SP-C have been monitored by measurements of the lifetime and anisotropy of exogenous fluorescent probes. The probe 1-palmitoyl-2-(6-(7-nitrobenzoxadiazol-4-yl)aminohexanoyl)phosphatidylcholine (NBD-PC) exhibits two well-resolved lifetimes in PL multilamellar vesicles. Incorporation of SP-C (1% w/w) modestly alters the proportions of the two lifetime components and the extent of their quenching by iodide. SP-C lessens the cooperativity of the gel to liquid crystal (l-c) phase transition and broadens the transition temperature breadth, as indicated by steady-state anisotropy. Measurements of polarization at multiple frequencies on a phase-shift fluorometer, which permit calculation of rotational correlation times, reveal that SP-C causes a large increase in the limiting anisotropy (r_∞) in both gel and l-c phases. Thus SP-C incorporation increases the PL order parameter.

M-Pos166 THE EFFECT OF 1,2,3-HEPTANETRIOL ON MICELLE SIZE.

C.-y. Hsu, J.F. Hunt, M. Kataoka, and D.M. Engelman, Dept. of Molecular Biophysics and Biochemistry, Yale University. (Introduced by Franklin Hutchinson.)

We have used small angle x-ray scattering and HPLC gel filtration chromatography to study the effect of the small amphiphile 1,2,3-heptanetriol on the size of β-octylglucoside (β-og) micelles in aqueous solution. Both techniques suggest a large reduction in the size of the micelle in the presence of the small amphiphile. Small angle x-ray scattering shows the radius of gyration (Rg) of the β-og micelle to be 28.7±0.5 Å in a 3% (w/v) solution; in the presence of 3%(w/v) 1,2,3-heptanetriol, the Rg is observed to fall to 25.0±0.7 Å, corresponding to a reduction in volume on the order of 35%. Control experiments using sucrose to vary the electron density of bulk solvent indicate that the observed decline in the Rg of the detergent micelle is unlikely to arise as an artifact of contrast variation. HPLC gel filtration experiments confirm the results of the small angle scattering experiments, showing a reduction of approximately 50% in the volume of the β-og micelle in the presence of the small amphiphile. Furthermore, gel filtration experiments have been used to study the effect of the small amphiphile on the size of mixed micelles containing either phospholipid or protein; these experiments reveal a 40% reduction in the size of DMPC/β-og mixed micelles and a 30% reduction in the size of mixed micelles containing a synthetic peptide based on the sequence of the interleukin-2 receptor transmembrane domain. The size of bacteriorhodopsin/β-og mixed micelles is reduced by a more modest amount, varying between 5% and 10% depending on salt conditions. As a consequence of these effects, 1,2,3-heptanetriol dramatically changes the resolution observed in gel filtration chromatography in β-octylglucoside and may extend the utility of this technique in the field of membrane protein purification. Moreover, the influence of the small amphiphile on the size of detergent micelles and protein/detergent mixed micelles may contribute to its beneficial effect in membrane protein crystallization reactions.

M-Pos167 THERMODYNAMICS OF INTERSUBUNIT INTERACTIONS IN CHOLERA TOXIN UPON BINDING TO ITS CELL SURFACE RECEPTOR, GANGLIOSIDE G_{M1}. Arne Schon and Ernesto Freire. Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

The energetics of the interaction of cholera toxin with the oligosaccharide portion of ganglioside G_{M1} (oligo-G_{M1}), the toxin cell surface receptor, has been studied by high-sensitivity isothermal titration calorimetry. Previously, we have shown that the association of cholera toxin to ganglioside G_{M1} enhances the cooperative interactions between subunits in the B-subunit pentamer. New experiments have revealed that the oligosaccharide portion of the receptor is by itself able to enhance the intersubunit cooperative interactions within the B pentamer. This effect is seen in the protein unfolding transition as a shift from independent unfolding of the B-protomers towards a cooperative unfolding. The binding of cholera toxin to oligo-G_{M1} has been measured calorimetrically as a function of the degree of saturation. The binding curve at 37° C is sigmoidal indicating cooperative binding. The binding data can be described in terms of a nearest neighbors cooperative interaction binding model. In terms of this model, the association of a oligo-G_{M1} molecule to a B-protomer affects the association to adjacent B-protomers. The intrinsic binding enthalpy was -25 kcal-mol and the cooperative interaction enthalpy -12 kcal/mol. The intrinsic binding constant was $1.25 \cdot 10^6$ M^{-1} and the cooperative Gibbs energy equal to -940 cal/mol. The same thermodynamic model can be used to describe the changes in protein fluorescence that accompany the binding of the toxin to oligo-G_{M1}. These studies provide the first direct thermodynamic description of the cooperative binding of a protein to its cell surface receptor. (Supported by NIH Grant NS-24520.)

M-Pos168 SPONTANEOUS RAMAN AND RESONANCE RAMAN STUDY OF THE INTERACTION OF FERRICYTOCHROME c WITH INTERDIGITATED AND PERTURBED PHOSPHOLIPID BILAYERS. *James S. Vincent[a] and Ira W. Levin[b], [a]Chemistry Department, University of Maryland Baltimore County, Catonsville, Maryland 21228, and [b]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.*

The vibrational Raman spectra of di-O-hexadecylphosphatidylcholine (DHPC) and dipalmitoylphosphatidylcholine (DPPC) liposomes reconstituted with ferricytochrome c under perturbed conditions, including interdigitation, are reported as a function of temperature. The DPPC bilayers are reconstituted with 100 mg ethanol per ml water to insure that ferricyctochrome c interacts with an interdigitated system. DPPC bilayers are also formed with 18% cholesterol and the protein. Total integrated band intensities and relative peak height intensity ratios, two spectral scattering parameters used to determine bilayer disorder, are sensitive to the presence of ferricytochrome c in the reconstituted liposomes. Protein concentrations were estimated by comparing the 1636 cm^{-1} resonance Raman line of known ferricytochrome c solutions to intensity values for the reconstituted multilayer samples. Temperature dependent profiles of the 3100-2800 cm^{-1} C-H stretching, 1150-1000 cm^{-1} C-C stretching, 1440 cm^{-1} CH_2 deformation and the 1295 cm^{-1} CH_2 twisting mode regions characteristic of acyl chain vibrations reflect bilayer perturbations due to the interactions of ferricytochrome c. The multilamellar gel to liquid crystalline phase transition temperature T_M, defined by spectral parameters, is altered for the ferricytochrome c reconstituted DPPC and DHPC liposomes compared with the pure lipid. Other spectral features are interpreted in terms of protein penetration into the hydrophobic region of the bilayer.

M-Pos169 THE ABILITY OF THE HIGH MOLECULAR WEIGHT LUNG SURFACTANT-ASSOCIATED PROTEIN, SP-A, TO PROMOTE LIPID MIXING OF SYNTHETIC LIPOSOMAL MEMBRANES. Kathleen Shiffer[1], Roberta A. Parente[2], Samuel Hawgood[3], and Jon Goerke[4] (Intr. by Francis Szoka, Jr., Ph.D). [1,3,4]Cardiovascular Research Institute, and Depts. of [2]Pharmacy and Pharmaceutical Chemistry, [3]Pediatrics, and [4]Physiology, University of California, San Francisco, CA 94143.

SP-A induced lipid mixing between two populations of egg phosphatidylglycerol large unilamellar vesicles (egg PG LUV) in the presence of calcium, as monitored by resonance energy transfer between two different fluorescent lipid probes, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) and N-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine (Rh-PE). Since the threshold level for lipid mixing of egg PG LUV by Ca^{2+} alone is 15 mM, the lipid mixing which occurred at Ca^{2+} concentrations between 1.5 and 3 mM was due to the addition of SP-A. At pH 6.9 and a phospholipid (PL)/SP-A ratio (w/w) of 3/1, Ca^{2+} concentrations of 1.5, 2, 2.5, 3, 15, and 30 mM produced 8, 18, 28, 42, 68, and 74 % lipid mixing, and at pH 6.9 and 3 mM Ca^{2+}, PL/SP-A (w/w) ratios of 10/1, 5/1, 3/1, and 2/1 produced 8, 20, 42, and 50% lipid mixing. Therefore, the extent of lipid mixing was related to both the Ca^{2+} and the SP-A concentrations. In the absence of Ca^{2+}, lipid mixing would only occur if the pH of the assay buffer was less than 5.0. In addition, SP-A did not induce lipid mixing of pure dipalmitoylphosphatidylcholine (DPPC) LUV in the presence or absence of Ca^{2+}, or at a pH less than 5.0. All experiments were conducted at 37 C.

Our data suggest a role for SP-A in the mixing of membrane lipids (i.e., in the formation of structures such as tubular myelin). This same protein has previously been shown to promote surface film formation, particularly in the presence of the low molecular weight surfactant-associated proteins, SP-B and SP-C. Supported by HL-07275 and NHLBI-24075.

M-Pos170 ENVIRONMENTAL EFFECTS ON THE STRUCTURE AND DYNAMICS OF M13 COAT PROTEIN (A MODEL MEMBRANE PROTEIN) USING ^{13}C NMR SPECTROSCOPY. Gillian D. Henry and Brian D. Sykes Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

The coat protein of the filamentous coliphage M13 is a 50-residue polypeptide chain which spans the inner membrane of the host *E. coli* cell duing the reproductive cycle of the phage. It comprises a 19-residue hydrophobic central core (which spans the lipid bilayer during infection) flanked by hydrophilic N- and C-terminal regions. The coat protein aggregates extensively in the absence of detergent or lipid. We have undertaken a comparative study of the coat protein in a variety of different solubilizing amphiphiles, most notably sodium dodecylsulphate (SDS), sodium deoxycholate and β-D-octylglucoside. The overall correlation times of these protein-detergent complexes are about 10 ns. Protein backbone motions were assessed from relaxation behaviour of various labelled carbonyl carbons. Although the spectra obtained in different detergents were quite similar, the linewidths differed markedly probably due to variations in conformational heterogeneity. The narrowest lines were obtained in SDS. Two resonances per labelled carbon site were observed for some labels in SDS-solubilized protein suggesting the protein to be an asymmetric dimer. Relaxation parameters (T_1 and nOe values) were also measured for a side chain, that of the single methionine (met-28) labelled with ^{13}C on the methyl group. The motional properties of met-28, a residue within the hydrophobic core of the protein, and its sensitivity to temperature and the nature of the solubilizing detergent will be discussed.

M-Pos171 SPECTRIN-INDUCED LEAKAGE OF SMALL MOLECULES FROM LIPOSOMES. Nanda K. Subbarao, Keizo Takeshita and Robert C. MacDonald. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University. Evanston IL 60208.

The nature of the direct interaction between spectrin and lipid on the red cell bilayer has been studied by examining the influence of spectrin on the leakage of small molecules from liposomes under physiological conditions. Detectable denaturation of spectrin was ruled out by the measurement of its near-UV circular dichroism, and spectrin was shown to be fully functional with respect to its dimer-tetramer equilibrium by gel electrophoresis under non-denaturing conditions. Leakage was assessed according to the extent of release of calcein entrapped at self-quenched concentrations in extruded vesicles in 100 mM NaCl, 5 mM Tris/HCl, 0.1 mM EDTA, pH 7.4, in 3 h at room temperature. At a lipid/spectrin dimer molar ratio of 3000:1, 26% of the liposome contents leaked from bovine brain phosphatidylserine (PS) vesicles, whereas 9% leaked from phosphatidylcholine (PC) vesicles. The presence of 2 mM magnesium or 0.5 mM calcium ions did not alter these induced leakages significantly. Spectrin induced 1/4 as much leakage from sonicated vesicles as from extruded ones. Heat-denatured spectrin (100°C, 10 min) retained only 1/3 as much leakage-inducing activity as did native spectrin. However, tryptic digests of the protein were as effective as the intact protein in inducing leakage from PS vesicles. The hydrophobicity of spectrin is too low for it to embed in lipid bilayers. Therefore, spectrin's ability to induce leakage probably reflects its capacity to bind to the head group region of the lipid.

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M-Pos172 THE RATE OF PENETRATION OF THE HYDROPHOBIC DOMAIN OF CYTOCHROME B_5 INTO THE BILAYER OF A LIPID VESICLE. N. Krishnamachary and Peter W. Holloway, Dept. of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.

The interaction of cytochrome b_5 with lipid vesicles has been shown to be a rapid, spontaneous reaction. The binding process can be monitored by observing the change in fluorescence of the tryptophans in the hydrophobic membrane-binding domain. When binding occurs to vesicles made from 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) there is a 1.65 fold increase in fluorescence. The kinetics of binding to POPC and to brominated lipid have been followed by means of stopped-flow fluorescence techniques. Cytochrome b_5 exists in aqueous solution as a mixture of monomer and octamer, and binding to vesicles occurs through the monomer form. When binding occurs to POPC vesicles at 25°C there is an immediate rise in fluorescence within the dead time of the instrument, followed by a slower rate of $k = 4.0$ sec^{-1}. The latter rate is the rate of dissociation of the octamer to monomer, which is followed by the rapid binding of monomer. When 1,2-(9,10-dibromostearoyl)phosphatidylcholine (BRPC) is used in a static experiment the tryptophan fluorescence is quenched to 39% of the value seen with POPC. When BRPC is examined by stopped-flow there is an immediate rise in fluorescence, to 84% of the value seen with POPC, followed by a very slow decrease ($k = 0.17$ sec^{-1}). This slow rate is reduced when the binding occurs at lower temperatures and when the bromine atoms are closer to the center of the bilayer. Inclusion of 30 mole% cholesterol in the vesicles also reduces the rate of quenching by the BRPC. In contrast to the slow quenching seen with the cytochrome, the binding of tryptophan octyl ester to BRPC is accompanied by an immediate quenching. We conclude that the slow phase quenching is the rate of penetration of the hydrophobic tail of the cytochrome into the bilayer. Supported by NIH Grant GM 23858

M-Pos173 **Membrane-Binding and Structure of a Spin-Labeled Alamethicin Derivative**

Sharon J. Archer, Jeffrey F. Ellena and David S. Cafiso, Department of Chemistry, University of Virginia, Charlottesville, VA 22901.

A spin-labeled derivative of the ion-conductive peptide alamethicin was synthesized by attaching 3-carboxyproxyl to the C-terminal phenylalaninol residue. Under conditions where the peptide partitions between membrane and aqueous phases, a two component EPR spectrum is observed. From the amplitude of these components, the partitioning of the peptide has been determined. This method is being used to investigate the electric field dependence of the alamethicin binding, a process that may be responsible for the voltage-dependence of this channel in planar bilayers. In egg PC vesicles, the membrane bound lineshape of the label is a strong function of the alamethicin concentration, and is likely the result of the aggregation of membrane bound alamethicin. 1H NMR spectroscopy of this peptide and 13C NMR spectroscopy of the membrane lipid provide additional information on the structure of alamethicin. The C-terminus of alamethicin is located near the carbonyl group of the membrane lipid, as indicated by paramagnetic effects on the 13C spin-lattice relaxation of the lipid nuclei. Using 1H NMR, paramagnetic effects on the 1D linewidths and intensities in 2D COSY spectra for the proxyl-alamethicin were measured. These effects provide information on the proximity of the C-terminus of the peptide relative to other residues on the peptide in solution and micelles.

M-Pos174 THE EFFECTS OF VISCOSITY ON GRAMICIDIN TRYPTOPHAN MOTIONS S.F.Scarlata Cornell Univ.Med.College 1300 York Ave., New York, NY 10021

The rotational amplitude of gramicidin tryptophans was investigated as a function of temperature and viscosity in a variety of solvents using fluorescence spectroscopy. In 80% glycerol-ethanol, gramicidin behavior was similar to that of alpha helical globular proteins. In dioleoyl-phosphatidylcholine (DOPC) and egg-phosphatidylcholine bilayers, the rotational amplitude of the tryptophans remained constant from 5 to 40°C due to the large number of tryptophans participating in intermolecular aromatic ring stacking. In gel phase dimyristoyl-phosphatiylcholine (DMPC), the tryptophan rotations likewise do not respond to temperature and viscosity changes, presumably because of a combination of Trp 9 and 15 stacking and the high viscosity of the membrane. In fluid phase DMPC, stacking becomes disrupted as the temperature increases causing the change in tryptophan amplitude with temperature to be greater than allowed by the membrane. In n-octylglucoside micelles, ring interactions are also broken with heat. Increasing the membrane viscosity isothermally by high pressure causes an increase in tryptophan rotational amplitude while decreasing the rotational amplitude of the fatty acid probes. We conclude that membrane viscosity regulates both inter- and intra-molecular gramicidin interactions but not in a straightforward manner.

M-Pos175 MEMBRANE INCORPORATION AND INDUCTION OF SECONDARY STRUCTURE OF SYNTHETIC SIGNAL PEPTIDES CORRESPONDING TO THE N-TERMINAL SIGNAL SEQUENCES OF THE GLUCITOL AND MANNITOL PERMEASES OF E.COLI. Lukas K. Tamm[1], John M. Tomich[2], and Milton H. Saier Jr.[3] (Intro. by R. Wohlgemuth) [1]Biocenter, University of Basel, CH-4056 Basel, Switzerland, [2]Medical Genetics Division, Childrens Hospital, Los Angeles, CA 90027, and [3]Dept. of Biology, University of California at San Diego, San Diego, CA 92093

The 22 residue synthetic signal peptide of the glucitol permease (enzyme II^{gut} of the bacterial phosphotransferase system) was found to insert into phospholipid monolayers of various phospholipid compositions up to limiting pressures above 36 mN/m. The partition coefficient, derived from monolayer area expansion measurements was, 1.0×10^5 for partitioning of the negatively charged peptide into monolayers of POPC. Gut-22 contains a titratable histidyl residue (pK_a=6.8) and its protonation decreased the relative monolayer area increase three-fold. Circular dichroism spectra showed that gut-22 formed an amphiphilic α-helix when incorporated into lipid membranes (estimated percent helix = 65%). Fluorescence measurements indicated that tryptophan 11 is in a more hydrophobic environment in the presence of lipid than in its absence, with the environment being more hydrophobic at pH 5 than at pH 8.

The more hydrophilic 15 residue signal peptide of the mannitol permease also incorporated into neutral and charged monolayers, but to a much lesser extent. It was a random coil in aqueous solution, but became partially structured when bound to SDS micelles. Based on these results and a parallel with mitochondrial targeting in eucaryotes, we suggest that the induction of N-terminal amphiphilic structures and their association with a hydrophobic-hydrophilic interface is important for the initiation of the membrane insertion of bacterial PTS permeases.

M-Pos176 STRUCTURE AND DYNAMICS OF DMPA/CA^{2+} AND DMPA/POLY-L-LYSINE COMPLEXES.

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We have studied the effect of calcium ions and of poly-L-lysines (PLL) of different molecular weights (M_W≈4 000 and M_W≈200 000) on dimyristoylphosphatidic acid (DMPA) bilayers by Raman, IR, ^{2}H-NMR spectroscopies and X-ray diffraction. Both vibrational and NMR spectroscopies show the importance of the degree of polymerization of PLL when it interacts with DMPA. Long PLL produces a 20°C shift to higher temperatures of the gel-to-fluid phase transition of the lipid. The NMR data clearly show that the order within the bilayer is increased in the fluid phase due to the presence of long PLL. By contrast, short PLL has no major effect on the gel-to-fluid phase transition temperature of the lipid even though the order of the lipid acyl chains is somewhat higher in the fluid phase. This different thermotropic behaviors of PLL/DMPA complexes are clearly explained by the Raman data which show that long PLL bound to DMPA adopts the β-sheet structure over the whole range of temperature investigated while the short polypeptide undergoes a change of conformation from β-sheet to random coil near 45°C. The effect of calcium ions on DMPA bilayers is quite different. Our results show that DMPA/Ca^{2+} complexes undergo a transition near 75°C which cannot be assigned to the $L_\beta \rightarrow L_\alpha$ transition.

M-Pos177 A QUANTITATIVE MODEL FOR THE BINDING OF SPECIFIC LIPID SPECIES TO EXTRINSIC MEMBRANE PROTEINS

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A new model is presented and quantitatively treated to explain the surface binding of certain extrinsic membrane proteins. The model presumes that there exists on such a protein molecule a limited number of binding sites specific for a sub-class of special lipids. Macroscopic binding affinity is determined by two processes: non-specific adsorption of protein to the membrane surface and association of a subclass of membrane lipids with the specific sites on the bound protein. Analysis of binding data is illustrated on several levels using data for the binding of two blood coagulation proteins, prothrombin and factor X/X_a, to synthetic membranes containing acidic phospholipids, either dioleoylphosphatidylglycerol or bovine brain phosphatidylserine (Cutsforth, Whittaker & Lentz, Biochemistry, submitted). On the simplest level, variation of the observed binding constant with the mol fraction of special lipid in the membrane yielded a rough estimate of the average number of molecules of lipid bound to sites on the protein. On a more precise level, analysis of Klotz plots of the data at low surface occupancy revealed that three acidic lipid binding sites were occupied on each bound protein. On another level, the model was treated exactly for the simple case of equivalent, non-interacting, protein-associated binding sites. It was necessary to adjust the values of only the non-specific adsorption equilibrium constant and the equilibrium constants describing binding of specific lipids to individual sites on the protein to obtain a good description of Klotz plots for a range of acidic lipid surface concentrations. We conclude that the simple model proposed here offers an adequate description of coagulation protein binding over a wide range of conditions and may be applicable to the binding of other similar extrinsic membrane proteins. Supported by HL26309 (The UNC SCOR in Thrombosis and Hemostasis) to BRL and by RR02736 to JH.

M-Pos178 INFLUENCE OF PROTEINS ON FLUOROPHORE LIFETIME DISTRIBUTIONS IN MEMBRANES.

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When the fluorescence decay of fluorophores in lipid bilayers is analyzed as a fluorescence lifetime distribution, the width of the distribution (f_w) reveals the degree of structural heterogeneity detected by the fluorophore. For diphenylhexatriene (DPH), in natural membranes containing proteins, the f_w was found to be greater than that found in vesicles of extracted lipids. This suggests that the fluorophores in the vicinity of the protein may detect a degree of structural heterogeneity greater than in the bulk lipid phase. In order to characterize this effect we have examined the fluorescence lifetime distribution of DPH in vesicles of 16:0/18:1-phosphatidylcholine (POPC) and dimyristoylphosphatidylcholine (DML) into which glycophorin and gramicidin D respectively had been reconstituted (at 37°C). Analysis of DPH in vesicles of POPC and DML alone showed a narrow f_w, indicating a relatively homogeneous probe environment. In contrast, the presence of the proteins gave a broad f_w (2-4ns) indicating the presence of structural diversity adjacent to the protein. In effect, this may allow spectroscopic isolatation of the protein-lipid interfacial region. We would expect this approach should be useful in understanding how drugs, anesthetics and ethanol might modify protein functioning by interactions at this region of the membrane.

M-Pos179 **INFLUENCE OF PROTEINS ON FLUOROPHORE LIFETIME DISTRIBUTIONS IN MEMBRANES: EFFECT OF QUENCHERS OF FLUORESCENCE.**

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Fluorescence lifetime distributional analysis of diphenylhexatriene (DPH) in membranes containing proteins reveal a broad distribution (f_w) compared to vesicles made from lipids alone. This suggests that the region around the protein may contain a structural diversity which is detected by the fluorophore. Membrane proteins often contain intrinsic quenchers of fluorescence, such as heme or retinal which might be expected to increase the f_w by fluorescence-energy transfer, due to the range of fluorophore-quencher distances. Studies with NBD-PE (quencher) and DPH (donor) in 16:0/18:1-phosphatidylcholine vesicles confirmed that this effect could produce a distributional width and that it would be of the order found for DPH in natural membranes (~2-3ns). To further investigate this effect we examined microsomal cytochrome b_5 and bacteriorhodopsin reconstituted into lipid vesicle systems. Both systems showed a decreased lifetime center (expected from fluorescence quenching) and a broad f_w, compared to the lipid vesicles without the proteins. On removal of the heme from cytochome b_5 and bleaching of the retinal, the f_w narrowed and the lifetime center returned to that found in the absence of proteins, confirming the contribution of fluorescence-energy transfer to the distributional width. The remaining distributional width was presumed to be due to the structural heterogeneity of the protein-lipid interfacial region.

M-Pos180 PHYSICAL DETERMINANTS OF INTERMEMBRANE PROTEIN TRANSFER. S. I. Waters, R. Sen, W. H. Huestis, L. S. Brunauer, Stanford University, Stanford, CA.

Spontaneous transfer of membrane proteins between cells and liposomes has been described for a number of cell systems. We have examined protein transfer under a variety of conditions to investigate physical factors governing this process. Human erythrocytes were incubated with sonicated dimyristoylphosphatidylcholine (DMPC) vesicles containing trace [14C]dipalmitoyl-phosphatidylcholine. Protein-vesicle complexes (PVs) were separated from cells and from membrane fragments by centrifugation. The yield of isolated PVs was determined from the 14C vesicle marker; protein compositions were analyzed by SDS-gel electrophoresis. Enzymatic removal of portions of the cytoplasmic or exoplasmic domains of cell membrane proteins had little effect on the extent or selectivity of protein transfer. Pronase treatment is expected to change charge character and steric blockage of the erythrocyte surface. Cells pretreated with pronase, however, did not differ significantly from controls in the quantity of band 3 susceptible to transfer. Trypsin cleavage of the cytoplasmic domain of band 3 also had no effect on the extent of its transfer from red cell membrane vesicles. However, the extent and selectivity of protein transfer from red cells was influenced by the lipid composition of the recipient vesicles. Inclusion of cholesterol increased the extent of transfer, and relative quantities of the transferred proteins differed for DMPC vesicles and for vesicles composed of DMPC/phosphatidylserine or DMPC/cholesterol mixtures. Protein transferred from 32P labeled cells differed in specific radioactivity from the bulk cell protein. Transferred band 3 exhibited higher specific activity than total cell band 3, while glycophorin, highly phosphorylated in the cell membrane, showed no detectable labeling in the corresponding PV gel band. These observations suggest that cell-to-vesicle protein transfer a) is insensitive to bulk steric and electrostatic properties of cell membranes, b) is influenced by selective affinities of proteins to particular lipids, and c) may reveal subtle differences in the membrane association of protein subpopulations.