M-Pos234 **LIPID-PROTEIN INTERACTIONS INFLUENCE THE COLOR OF BACTERIORHODOPSIN.** C. Pande*, P. Rath*, R. Callender*, A. Pande+, J. Baribeau++ and F. Boucher++. *Physics department, City College of New York, U.S.A.; +Universitatsspital, Zuerich, Switzerland; ++Centre de Recherche en Photobiophysique, Universite du Quebec a Trois-Rivieres, Quebec, Canada.

Detergent solubilization and subsequent delipidation of bR results in the formation of a new species absorbing maximally at 480 nm (bR_{480}) in a pH dependent equilibrium with the parent species. The pK of this transition is sensitive to the detergent which replaces the lipids (Baribeau and Boucher, unpublished) and in the limit of detergent free, lipid free, pigment its value is 2.6, with higher pH favoring the bR_{480} form. Resonance Raman spectroscopy shows that, like the native bR, bR_{480} also contains a protonated Schiff base linkage between the chromophore and the protein. However, the Schiff base vibrational frequency in the bR_{480}, as well as its shift upon deuteration, are quite different from those in the native bR suggesting changes in the Schiff base environment upon delipidation.

We interpret these results to suggest that some protein residue whose pK is sensitive to the lipid-protein interactions is responsible for the observed transition. The pK changes are presumably related to protein structural changes that may result from the loss of lipid-protein interactions upon delipidation. These changes appear to affect the Schiff base region in particular, causing electronic perturbations in the chromophore which result in the observed hypsochromic shift in the absorption maximum. FTIR and CD studies are being persued to explore these changes.

M-Pos235 MOLECULAR DYNAMICS OF THE PRIMARY PHOTOCHEMICAL EVENT IN BACTERIORHODOPSIN Leonore A. Findsen, Brian M. Pierce and Robert R. Birge Department of Chemistry, Carnegie-Mellon University, Pittsburgh, PA 15213

The nature of the primary photochemical event in light adapted bacteriorhodopsin ($bR_{568} \rightarrow K_{610}$) is studied using semiempirical molecular dynamics theory. This study uses Intermediate Neglect of Differential Overlap including Partial Single and Double Configuration Interaction (INDO-PSDCI) for the calculation of the potential surfaces. We predict theoretically that the photoisomerization forming K_{610} from bR_{568} is accomplished in ~0.8 ps. with a calculated quantum yield of 0.27. The latter is in reasonable agreement with the experimental value of 0.33 [Hurley and Ebrey, *Biophys. J.*, 22, 49(1978)]. In addition, simulations of the primary photochemical event of bR_{568} indicate that ~39% of the excited state species are trapped in a potential minimum not coupled dynamically to the ground state surface. This population will radiationlessly decay to reform bR_{568} (not K_{610}) and we propose that absorption of light by these trapped excited state species may be responsible for the appearance of J_{625} [Ippen, *et al.*, *Science*, 200, 1279(1978)], a spectroscopic intermediate which precedes the formation of K_{610}.

M-Pos236 TWO-PHOTON SPECTROSCOPY OF RHODOPSIN AND BACTERIORHODOPSIN Mark M. Masthay, Chian-Fan Zhang, G. Alan Schick, Henry M. Knapp, and Robert R. Birge, Department of Chemistry, Carnegie-Mellon University, Pittsburgh, PA 15213

Two-photon thermal lens spectra of rhodopsin and two-photon double resonance spectra of bacteriorhodopsin were taken with the goal of locating the low-lying, covalent $^1A_g^*$-like $\pi\pi^*$ states in the protein bound chromophores. (Because of the photochemical lability of rhodopsin, a model rhodopsin containing a locked 11-*cis* chromophore was used.) The two-photon spectra indicate that the binding sites of both proteins are neutral, and contain a protonated Schiff base chromophore and one or two nearby counterions with a net total charge of -1. The sensitivity of the energy splitting of the low-lying "allowed" and "forbidden" $\pi\pi^*$ states to small changes in counterion location provides a spectroscopic window on the local counterion environment. Models of the rhodopsin and bacteriorhodopsin binding sites which accommodate our spectroscopic data will be presented. The large two-photon absorptivity of the 410 nm band in light-adapted bacteriorhodopsin indicates that it is either "$^1A_g^{*+}$" or "$^1B_u^{*-}$" in character. The latter assignment would indicate a 6-s-*cis* geometry for the polyene chromophore since the two-photon absorptivity of the "$^1B_u^{*-}$" state is enhanced by *cis*-linkages. One-photon and two-photon polarization studies are in progress at this time to provide a definite assignment to this band.

M-Pos237 LIGHT-INDUCED POLARIZED FOURIER TRANSFORM INFRARED SPECTROSCOPY OF BACTERIORHODOPSIN - A STUDY OF THE M412 INTERMEDIATE BY PHOTOSELECTION -

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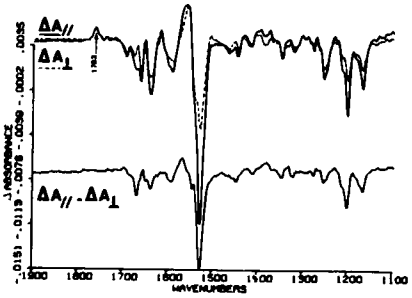
The orientation of the infrared (IR) transitions of bacteriorhodopsin (BR) and its M412 intermediate relative to the optical transition of the retinal in BR (568 nm) has been investigated by light-induced Fourier transform IR difference spectroscopy using a photoselection technique on films of oriented purple membrane. Both the IR beam and the visible excitation beam propagated along the normal to the film plane. The IR beam was polarized parallel ($\Delta A_{//}$) or perpendicular ($\Delta A_{\perp}$) to the polarization of the excitation beam. This geometry allows to detect the angle θ between the projections on the film plane of a given IR transition and of the retinal optical transition. The BR C=C and C14-C15 retinal vibrations at 1527 cm^{-1} and 1200 cm^{-1}, respectively show a dichroic ratio of 2, demonstrating that θ=0°. The M412 C=C vibration at 1565 cm^{-1} is significantly less dichroic. The 1762 cm^{-1} C=O stretching vibration, assigned to an aspartic carboxyl group which becomes protonated in M412, is not dichroic, indicating a 45° θ value for this transition. Together with the azimutal angles measured by IR dichroism (Nabedryk and Breton, 1986, FEBS Lett. 202, 356-360), this measurement reveals the relative angles between the 568 nm optical transition and several IR transitions in BR and M412.



M-Pos238 POLARIZED FTIR INVESTIGATION OF SECONDARY STRUCTURE IN ORIENTED BACTERIORHODOPSIN. W.K. Liddle, R.B. Dyer, N.W. Downer, W.H. Woodruff and Jill Trewhella, Life Sciences Division, Inorganic Nuclear Chemistry Division, and Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM 87545.

There is a continuing controversy concerning whether or not bacteriorhodopsin has a significant β-sheet component in its transmembrane regions. Resolution of this controversy is likely to have some impact on attempts to take the current low resolution model of bacteriorhodopsin (showing 7 transmembrane segments) to higher resolution. To date there has been a prevailing tendency to model the structure assuming purely α-helical composition. Earnest and Rothschild (1) reported on polarized FTIR studies of oriented purple membranes and concluded that there was a significant β-sheet component, while Nabedryk et al. (2) concluded from their polarized FTIR studies that there was not. We have undertaken similar studies in an attempt to resolve this discrepancy. We have measured polarized FTIR spectra from films of purple membranes that had been oriented by centrifugation. Films were made either from membranes in H_2O or membranes partially exchanged in D_2O so that faster exchanging peptide groups, presumably those in the extramembranous linker regions, would not contribute to the amide II band. Data were taken with vertically and horizontally polarized beams at varying angles of incidence to the membrane plane. Intensity changes in the amide I and amide II regions of the spectra will be interpreted in terms of the secondary structure content and the orientation of secondary structure elements in transmembrane regions of bacteriorhodopsin.

(1) Earnest and Rothschild, Biophys. J. (1986) 49:294a.
(2) Nabedryk et al., Biophys. J. (1985) 48:873.

This work is supported by DOE/OHER. Project #HA-02-02-03/B04664

M-Pos239 FTIR STUDY OF CARBOXYLIC ACIDS IN THE BACTERIORHODOPSIN PHOTOCYCLE

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Koji Nakanishi, Kazunori Odashima, Katsuhiro Konno ,and Weidong Ding, Dept. of Chemistry, Columbia University, New York, NY 10027

Infrared spectroscopy has revealed involvement of carboxylic groups in the bacteriorhodopsin (bR) photocycle. Some prominent bands in 1740-1760 cm^{-1} region were assigned to aspartic acid (Asp) COOH mode by ^{13}C labelling on Asp. Unable to resolve isotopic shifts of small features in 1730-1740 cm^{-1} region in K/bR and M/bR difference spectra led to attempts to assign them to glutamic acids (Glu) (Engelhard et al. 1985; Dollinger et al., in press). In the current study we measured bR with [5-^{13}C] Glu and [4-^{13}C] Asp (80% incorporated) by FTIR difference spectroscopy at low temperature. We could detect no shifts due to ^{13}C Glu in bR and K,L and M intermediates, but clear shifts of all features in carxyl region by ^{13}C Asp. It is conclusive that Glu does not contribute to any observed carboxylic group changes subject to protonation state or enviorenmental variation. This result affords an unambiguous distinction between the roles of two kinds of carboxylic acids and will simplify the mechanism of protein participation in bR photocycle and proton translocation. The previous assignments of Glu could be addressed to low ^{13}C incorporation ratios. This work was supported by HEW PHS 32455 (LE) and NSF CHE 84-12513 (KN).

M-Pos240 CONFORMATIONALLY DEPENDENT CHROMOPHORE / PROTEIN COUPLING IN BACTERIORHODOPSIN: TYROSINE PROTONATION STATE IN BR_{570}, BR_{548}, K_{630} AND C_{610}

Paul Roepe, Judith Herzfeld, Johan Lugtenburg and Kenneth Rothschild, **Dept. of Physics, Boston University, Boston, MA (P.R., K.R.), Dept. of Chemistry, Brandeis University, Waltham, MA (J.H.), Dept. of Chemistry University of Leiden, Leiden, The Netherlands (J.L.)**

Isotopically labelled tyrosines selectively incorporated into bacteriorhodopsin (bR) in combination with Fourier transform infrared (FTIR) difference spectroscopy has previously been used to identify tyrosine vibrations in the "light adapted" (BR_{570}) to "dark adapted" (BR_{548}) difference spectrum which indicate that a tyrosinate group protonates upon dark adaptation at 37°C (Roepe *et al.*, (1986), Biophys. J. 49, 479a). In this abstract, the primary phototransition of the BR_{548} photocycle, $BR_{548} \longrightarrow C_{610}$, at 81°K, is studied and compared to the primary phototransition of the BR_{570} photocycle, $BR_{570} \longrightarrow K_{630}$. It is found no tyrosine protonation change occurs in the $BR_{548} \longrightarrow C_{610}$ reaction, in contrast to the tyrosinate protonation seen in the $BR_{570} \longrightarrow K_{630}$ reaction (Rothschild *et al.*, PNAS 83, 347). The similarity in frequency and intensity of the tyrosine peaks in the $BR_{570} \longrightarrow BR_{548}$ and $BR_{570} \longrightarrow K_{630}$ difference spectra indicates that the tyrosine protonation changes in the two reactions most likely originate from the same residue. The absence of intense retinylidene hydrogen out of plane (HOOP) lines in the $BR_{548} \longrightarrow C_{610}$ difference spectrum indicates that the C_{610} chromophore is in a much less strained conformation than the K_{630} chromophore. However, since the tyrosine is protonated in both K_{630} and C_{610}, this relaxation cannot be related to the protonation state of the tyrosine. Examination of the effects of isotopic labelling on the C_{610} chromophore and comparison to prior results (S.O. Smith *et al.*, PNAS, 81, 2055) indicates formation of C_{610} from BR_{548} does not involve C=N bond isomerization. This implies isomerizations about both the $C_{13}=C_{14}$ and $C_{15}=N$ bonds in BR_{548} are linked to tyrosinate formation in BR_{570} which is required for subsequent proton pumping. (This work was supported by grants from the NSF to K.J.R, the NIH to J.H., and the ZWO to J.L.)

M-Pos241 POLARIZED FTIR-DIFFERENCE SPECTROSCOPY OF BACTERIORHODOPSIN

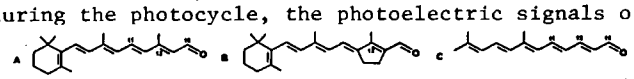
Thomas N. Earnest, Paul Roepe, Mark S. Braiman, and Kenneth J. Rothschild, Departments of Physics and Physiology, Boston University, Boston, MA 02215.

We have used low temperature, polarized FTIR-difference spectroscopy to investigate the orientation and conformation of protein and chromophore components of bacteriorhodopsin from the purple membrane of *Halobacterium halobium* in the light adapted state, bR_{570}, and its K_{630} and M_{412} photointermediates at 81 K and 250 K, respectively (Earnest *et al., Biochemistry*, 1986). Analysis of the IR linear dichroism of bands corresponding to C=C and C-C stretching modes of the retinal chromophore as well as the C=N Schiffs base stretch in the $bR_{570} \rightarrow K_{630}$ difference spectra yields an angle for the long axis of the polyene chain of 75° - 80° from the membrane normal. In contrast, upon warming to 250 K the angle between the polyene chain and the membrane normal decreases to 65° - 70°, in close agreement with the angle found for the electronic transition moment at room temperature. In addition, a small increase in the angle between the α-helices and the membrane normal is detected as the temperature goes from 81 K to 250 K. The dichroism of the "A_u" hydrogen-out-of-plane (HOOP) wags indicates that these modes lie mostly parallel to the membrane plane suggesting that the polyene plane is approximately perpendicular to the membrane plane at 81 K and 250 K. The orientation of transition dipole moments from protein components can also be deduced. Changes in the amide I (C=O stretch) region during the $bR_{570} \rightarrow K_{630}$ transition suggest alterations in the hydrogen bonding or orientation of one or more α-helices. For the $bR_{570} \rightarrow M_{412}$ transition, we observe bands at 1692 cm^{-1} and 1671 cm^{-1} arising from transition dipole moments which are oriented predominantly out of the plane of the membrane, whereas bands at 1658 cm^{-1} and 1639 cm^{-1} correspond to modes with transition dipole moments oriented mostly in the membrane plane. This dichroism is likely related to small localized orientational changes in amide groups of the peptide backbone. We also find the transition dipole moment angle of the C=O bond of an aspartic acid which is protonated between bR_{570} and M_{412} to be ≈ 43°. Supported by NSF grants PCM-8212709 and DMB-8509857 to KJR. Present address for TNE is Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, CA 94143.

M-Pos242 FAST PHOTOELECTRIC RESPONSES FROM ARTIFICAL PIGMENTS OF BACTERIORHODOPSIN

S. Liu [*], T. Ebrey [*], J. Zingoni [+], R. Crouch [+], J.-M Fang [**], and K. Nakanishi [**], [*]Department of Physiology and Biophysics, University of Illinois, Urbana, Il; [+]Opthalmology, Medical University of South Carolina, Charleston, SC; [**]Department of Chemistry, Columbia University, New York, N.Y.

The photovoltage signal of bacteriorhodopsin (BR) has at least three components with different lifetimes in the μs to ms range: a fast component (I) in the direction opposite to that of physiological proton translocation with lifetime around 10μs; a slow component (II) in the same direction as proton translocation with the lifetime around 0.1 ms; and a slower component (III) in the same direction as component II and with a lifetime around 8 ms. In order to understand charge movements during the photocycle, the photoelectric signals of BR with modified retinal chromophores were studied:



Analogue B's structure is very close to BR's normal chromophore (A). The pigment regenerated from B has similar λmax to BR but it cannot isomerize to a 13-cis structure as A does during the photocycle. Analogue C does not have the methyl group at the 13 position and lacks the ionone ring but it can isomerize at 13 position of A. The pigment regenerated from C has a broad spectrum with λmax around 525 nm. The kinetics of the photoelectric signal from all 3 membranes were measured in the μs to ms range with the membranes oriented in the polyacrylamide gels. Analogue B did not have any photoelectric response. Analogue C had the photoelectric response similar to component I and II of BR, but the ratio of II/I is only 1/3 of that seen for BR. These results suggest that component I is related to the charge movement induced by the isomerization of the retinal at 13 position. If component II is related to proton pumping, then analogue C pumps protons less efficiently than BR.

M-Pos243 PHOTOCYCLE OF NITRATED TYROSINE BACTERIORHODOPSIN R. Govindjee, Department of Physiology and Biophysics, University of Illinois, Urbana, Il 61801 and M. Tsuda, Department of Physics, Sapporo Medical College, Sapporo 060 Japan.

Nitration of tyrosine 64 in bacteriorhodopsin (BR) with tetranitromethane in light at pH=6.0 causes the absorption maximum to shift from 568 to 535 nm. The light-induced difference spectrum of the nitrated BR shows a decrease in absorbance centered around 535 nm and an increase in absorbance at 400 nm in the millisecond time scale (P. Scherer and W. Stoeckenius, Biochemistry 24, 7733, 1985).

In addition to the above mentioned absorbance changes we provide evidence for the presence of another intermediate with a maximum around 330 nm at pH=5.5. The half-decay time of this intermediate is approximately 150 ms which is much longer than even the slow phase of the decay of ΔA400, $\sim$40ms. The recovery of the bleaching at 510 nm also has a very slowly decaying phase which is slower than the slow phase in the decay of ΔA400 and is similar to the decay of the 330 nm photointermediate. It is likely that the 330 nm photointermediate is the same as R350 observed in native BR at high pH (see abstract, this meeting:"A new intermediate in the photocycle of bacteriorhodopsin"by Z.Dancshazy, R. Govindjee, B. Nelson and T.G. Ebrey).

M-Pos244 **Inhomogeneous Broadening in the Absorption Spectrum of Bacteriorhodopsin.**
David Braunstein, Mi Kyung Hong, Shuo-Liang Lin, Pál Ormos and Joseph Vittitow, Departments of Physics and Biophysics, University of Illinois, Urbana, IL 61801.

The inhomogeneous broadening of the spectrum of bacteriorhodopsin is assumed to be due at least partly to the conformational heterogeneity of the protein through the heterogeneity of protein-retinal interactions. We have used high-resolution fourier transform infrared difference spectroscopy to demonstrate the existence of this type of inhomogeneous broadening and to study the specific sources of heterogeneity.

In the temperature range 10–300K difference spectra of bR and the corresponding intermediate were taken following illumination with light of different wavelengths. Below 170K the spectra change with changing illumination wavelength, the most prominent feature being the shift of the ethylenic peak. Different wavelengths excite different parts of the heterogeneous bR population (a hole-burning effect).

According to a simulation model where a linear correlation of the ethylenic and visible absorption positions is assumed, about 10% of the total absorption bandwidth is due to this inhomogeneous broadening.

Studies on bR with isotope-modified retinal show that the heterogeneity responsible for the detected effect is located near the C_{10-11} bond in the chromophore.

M-Pos245 IDENTIFICATION OF AMINO ACIDS IN BACTERIORHODOPSIN INVOLVED IN INHIBITING FORMATION OF THE BLUE MEMBRANE. L. Brogley and R. Renthal (Intr. by D. Senseman), Univ. of Texas at San Antonio, San Antonio, TX 78285

Purple membrane (PM) converts to a blue pigment in acid or when divalent metals are removed. We previously showed that the acid blue membrane formation is inhibited by reaction of PM with 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC), but not when reacted in the presence of divalent metals. This suggests that the EDC modification of bacteriorhodopsin (BR) is at a residue(s) important in blue membrane formation and divalent metal binding. PM sheets (14 uM) were allowed to react with EDC·HCl (15 mM) in 36 mM NaCl at 5°C for 3 hr in the dark, after which the reaction was terminated with excess formic acid, the membrane delipidated, the BR cleaved with CNBr, and the fragments separated by HPLC. The major difference in the peptide map of EDC-modified BR compared with unmodified BR is the disappearance of half the peptide containing residues 69-118 and the quantitative appearance of a new peptide with the same amino acid composition. By comparison with our previously characterized modifications of this same peptide, the possible EDC-reactive sites may be limited to Asp 96, 102 and/or 104. These residues are thought to be located near the cytoplasmic surface of the membrane. It has been suggested that blue membrane formation involves neutralization of the counter-ion to the protonated Schiff base that links the retinal chromophore to BR (probably near the center of the membrane). If the EDC site is linked to the Schiff base counter-ion by a hydrogen-bonded network (proton wire), this could explain how modification of the membrane surface affects a site at the interior. (Supported by Am. Heart Assoc. Texas Affiliate, Welch Foundation, and NIH)

M-Pos246 ALTERED PROTEIN-CHROMOPHORE INTERACTIONS IN BACTERIORHODOPSIN MODIFIED AT ASP 115 BY DCCD. J. Vila and R. Renthal, Univ. of Texas at San Antonio, San Antonio, TX 78285

In detergent solution containing excess retinal, bacteriorhodopsin (BR) undergoes a light-dependent reaction with dicyclohexylcarbodiimide (DCCD), resulting in the conversion of aspartic acid 115 to the N-acyl dicyclohexyl urea (DCU) (Renthal et al., Biochem. 24:4275, 1985). We have further examined the properties of this modified BR. DCU-Asp 115-BR lacks the purple chromophore, but bleaching does not occur as a result of gross denaturation of BR: CD spectra in the 210-225 nm region are nearly the same before and after the DCCD reaction. The cyclohexyl rings of DCCD are required for the reaction: diisopropyl carbodiimide does not yield a bleached product and appears to modify a different site. In order to study the absorbance spectrum of DCU-Asp 115-BR, we sought conditions for reaction of purple membrane (PM) sheets with DCCD. At pH 7 in ether-saturated buffer, PM (10 uM BR) undergoes a light-dependent reaction with DCCD (180 uM). After 1 hr, unreacted DCCD is extracted with hexane. Peptide mapping indicates that the major site of modification is at Asp 115, just as in detergent. The absorbance spectrum of DCU-Asp 115-BR PM sheets has a maximum at approx. 380-400 nm (ε_{390}=20,000 $M^{-1}cm^{-1}$). The spectrum in this region is nearly identical before and after the reaction, while the 570 nm absorbance is virtually abolished. The loss of the 570 nm band cannot be due to the release of retinal from the protein: no retinal is extracted into the hexane wash after the reaction. Thus, the bleaching is probably due to alteration of protein-chromophore interactions by insertion of the DCU group at Asp 115. (Supported by grants from Am. Heart Assoc. Texas Affiliate, Welch Foundation, and NIH)

M-Pos247 COMPARATIVE CIRCULAR DICHROISM SPECTROSCOPY OF BACTERIAL RHODOPSINS. C.A.Hasselbacher[a], T.G. Dewey[b], and John L. Spudich[a,c] [a]Dept. Anat. Struct. Biol. and [c]Dept. Physiol. and Biophys., Albert Einstein Coll. of Med., Bronx, NY 10461, [b], Dept. Chem., Univ. Denver, Denver, CO 80208.

The structures of three protein pigments from Halobacterium halobium, bacteriorhodopsin (BR), halorhodopsin (HR), and sensory rhodopsin-I (SR-I), have been compared for a variety of conditions using CD spectroscopy. BR in purple membrane was compared to octylglucoside-purified HR after dialysis to remove detergent. HR and BR share similar features in all spectral regions from 190 to 700 nm. CD spectra between 190-240 nm indicate similar secondary structures for both proteins. A 315 nm negative band present in both spectra is less intense in the HR spectrum, which may indicate less rigidity in retinal-apoprotein interactions in HR. An HR bilobe is observed in the visible region of the spectrum and is symmetric about the absorbance maximum rather than asymmetric as is observed in BR. Low chloride converts the HR spectrum to one resembling that of BR. The HR spectrum in the visible region was analyzed using either an exciton dimer or trimer model. A map of possible configurations of the retinal within the protein is determined for each model. Blue-shifted M-like states of HR, in which the Schiff base is presumably deprotonated, can be generated by sustained illumination with orange light or by incubation in low chloride and high pH (Lanyi, Ann. Rev. Biophys. Biophys. Chem. 15, 11-28 (1986)). We have begun to compare the CD spectra obtained for these states to each other and to the CD spectrum of BR's M state which has been resolved previously (Draheim and Cassim, Biophys. J. 47, 497-507 (1985)). The visible CD spectrum of membranes containing SR-I as the only photoactive pigment as detected by flash photolysis exhibits a single positive band centered at the SR-I absorbance maximum.

M-Pos248 PIEZOELECTRIC ENERGY TRANSDUCTION IN ALPHA-HELICAL PROTEINS.
Roger E. Clapp, The MITRE Corporation, Bedford, MA 01730.

Three examples of biological energy transduction are examined: (1) the proton pump in bacteriorhodopsin, (2) the visual response in rhodopsin, and (3) the gating of the sodium ion channel. Each example is interpreted in terms of a piezoelectric process in one or more transmembrane α-helices. In this process two charges cross the membrane along two of the three chains of hydrogen bonds in the α-helical backbone; the straight α-helix is thereby changed into a curved α"-helix. In examples (1) and (2) the absorption of light forces the curving and drives the charges across; in example (3) the charge motion curves the helices, first opening and then closing the ion channel. To prove the mechanism in bacteriorhodopsin, one would illuminate the purple membrane of Halobacterium halobium in deuterated or tritiated water. One could then establish the locations in the membrane of those heavy protons (deuterons or tritons) that are in transit across a protein molecule. With the proposed mechanism, these atoms should replace the original H atoms along two chains of hydrogen bonds in an α-helical backbone. Their presence would be apparent through changes in the amide A, amide I, and amide II resonances, observable through Fourier transform infrared (FTIR) difference spectroscopy. (This experiment suggested by Henry Lester, Caltech, and by Dennis Mead, Columbia.) It should also be possible (perhaps with sealed vesicles) to count the number of light flashes that will fill some of the transmembrane queues and start delivering heavy protons to the other side of the membrane; this mechanism would appear to need at least eight or nine flashes, corresponding to the approximate number of tiers in a transmembrane alpha-helix. (This experiment suggested by Robert Stroud, UC San Francisco.)

M-Pos249 THE INVOLVEMENT OF CHARGED GROUPS IN BACTERIORHODOPSIN IN CATION BINDING Eva Hrabeta-Robinson and Lester Packer, Membrane Bioenergetic Group, Applied Sci. Div., LBL, Univ. of California, Berkeley, CA 94720

COOH groups in selective regions of bacteriorhodopsin (bR) were chemically modified and studied to determine the effect on cation binding. Visible spectroscopy and ESR were used to follow Mn++ binding. Mn++/bR ratios imply the need for a negative charge on the surface COOH groups in the binding of two cations and suggest that at least one cation is bound by interior COOH groups. Fractional conversion plots of the transitions reveal differences between the type of cation used and COOH modification performed. Modification of internal COOH groups to a neutral moiety enhances the heat-induced formation of blue species. Similarly, modification of surface COOH groups to a more acidic group results in enhanced blue species formation and, additionally, increased sensitivity to heat. Internal and external cross-linking and conversion of external COOH groups to a neutral group inhibit blue species formation at higher temperatures and result in a more labile protein. Thus, conformational mobility is required in blue species formation. Unmodified blue bR converted to purple by Na+ or Mn++ reforms a large amount of blue species and exhibits higher sensitivity to heat compared to bR with the normal compliment of cations. Ca++ reconstituted purple bR also differs in temperature sinsitivity from native bR. These observations indicate that a particular conformation is induced by the binding of a specific compliment of cations.

The binding of positively charged spin labelled amphophiles (CAT) at the same ratio as divalent cations to blue bR regenerates purple color. Studies of the M412 photocycle intermediate formed under steady state conditions show increasing the chain length of the CAT probes used to regenerate purple color results in accumulation of M412. Support by the Office of Biological Research, DOE, USA

M-Pos250 LARGE SCALE GLOBAL CONFORMATIONAL CHANGES INDUCED IN THE BACTERIO-RHODOPSINS OF THE PURPLE MEMBRANE BY LIGHT REDUCTION N.J. Gibson and J.Y. Cassim, Department of Microbiology and Program in Biophysics, The Ohio State University, Columbus, Ohio 43210

Past investigations in this laboratory into the nature of the retinal-apoprotein interactions in bacterio-rhodopsin (bR) during the photocycle have shown that 1) the excitonic CD bands disappear, indicating that the orientations of the retinals of the bR have randomized relative to each other, 2) the retinals have become more constrained, as evidenced by the appearance of fine structure in absorption and CD spectra, and 3) some of the helical segments of the bR within the membrane bilayer have tilted away from the membrane normal. In this study, we have attempted to learn more about these interactions by studying the reduced bR spectra. When bR is reduced with $NaBH_4$ in the presence of light ($\lambda > 450$ nm), the spectra show fairly featureless absorption and CD bands at 361 nm with no evidence of excitonic contributions. Films made with this preparation give similar results, but show a dramatic series of changes when briefly exposed to 361 nm light: 1) The band at 361 nm develops a greatly increased optical activity and fine structure, suggesting a more constrained retinal environment. The energy of this CD band is red-shifted by 10 nm relative to the solution and film absorption and CD bands seen when bR is reduced in the presence of light including $\lambda < 450$ nm. 2) The near-UV CD spectrum, which resembles that of bleached and M412 open bR films, becomes similar to that of the native closed bR form. 3) The far-UV CD spectrum suggests that the helical segments transform from a tilted open form to a more closed one in which the helices are more nearly normal to the membrane plane. These changes are reversible and seem to be regulated by the retinal, giving added evidence for the metastable nature of bacteriorhodopsin.

M-Pos251 THE PINK MEMBRANE, THE STABLE PHOTOPRODUCT OF DEIONIZED PURPLE MEMBRANE. Chung-Ho Chang*, Suyi Liu, Roy Jonas, and Rajni Govindjee. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801. *Present address: Department of Cell Biology, Stanford University, Stanford, CA 94305.

When divalent cations are removed from the purple membrane of Halobacterium halobium it turns blue (λ_{max} = 603 nm); this transition is facilitated by the removal of the last 20 C-terminal amino acids of bacteriorhodopsin. Continuous irradiation of this deionized blue membrane with intense red light ($\lambda \geq 630$ nm) converts it into a pink membrane (λ_{max} = 491 nm). The rate and extent of this transformation from the blue to the pink membrane also depends on the presence of the C-terminal amino acids. While the chromophore of the blue membrane is a 32:68 mixture of 13-cis and all-trans isomers of retinal, the chromophore of the pink membrane is 9-cis retinal. The quantum efficiency of the pink to blue membrane transition is relatively high as compared to that of the blue to pink photoconversion. A proton(s) is released when the pink membrane is converted to the blue form, but is taken up during the reverse transition. Unlike blue membrane, the pink membrane is unaffected by cation addition at sufficiently low pH and ionic strength.

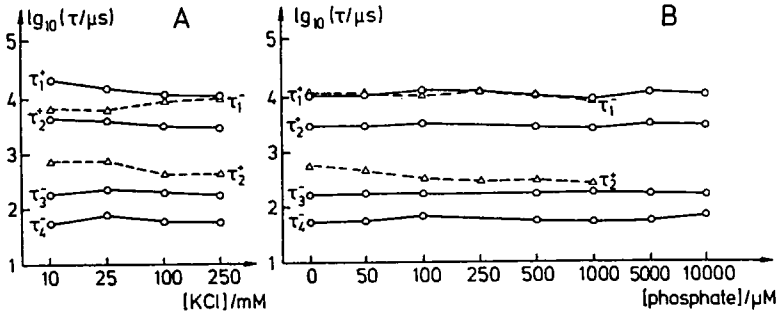
M-Pos252 MICELLAR SIZE AND STABILITY OF BACTERIORHODOPSIN MONOMERS IN ALKYLGLUCOSIDES. Charlotte B. Barnette[a], Larry J. DeLucas[b] and Donald D. Muccio[c]. Departments of Physiological Optics[a], Optometry[b] and Chemistry[c], University of Alabama at Birmingham, Birmingham, Al. 35294.

The micellar size of bacteriorhodopsin (bR) in octylglucoside was determined previously (1). In this study, the influence of the chain length of alkyl glucosides was investigated by size-exclusion HPLC. A comparison of the elution profiles of bR solubilized with heptyl-, octyl-, nonyl- and decylglucoside showed a systematic, but nonlinear, relationship between the monomeric micellar size and alkyl chain length (decyl>nonyl>octyl>heptyl). In addition to the monomeric peak, other minor peaks corresponding to higher aggregates were apparent in each elution profile. A peak occurred near the void volume for heptyl-, octyl- and nonyl-solubilized bR, which corresponds to 7-mers and larger. In contrast, the elution profile for decyl-solubilized bR showed a smaller aggregate, and that of the heptyl-solubilized bR contained an additional peak corresponding to bacterio-opsin (bO), suggesting that these bR micelles are less stable. The heat stability of each micellar prepartion was determined spectroscopically to also increase with the size of the alkyl chain length.

(1) D. D. Muccio and L. J. DeLucas, J. Chromatog. 326 (1985) 243-250.

M-Pos253 KINETICS AND STOICHIOMETRY OF LIGHT-INDUCED PROTON RELEASE BY PURPLE MEMBRANES. Stephan Grzesiek and Norbert A. Dencher, Biophysics Group, Department of Physics, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33, FRG.

The kinetics and stoichiometry of light-induced proton release in purple membrane suspensions have been investigated using the pH-indicator dye pyranine and single-turnover flash spectroscopy at a time resolution of 0.1 µs. The number of protons detected by pyranine is inversely proportional to the buffering power of the medium and 1.1 +/- 0.2 protons are released per photocycling bacteriorhodopsin molecule (pH 7.0, 20°C). Figs A/B depict time constants of exponential fits to formation and decay of the intermediate M-412 (solid lines) and protonation and deprotonation of pyranine (broken lines). Clearly, increasing amounts of phosphate buffer (B) or salt (A) accelerate the detection of the released protons, whereas the reuptake by the purple membrane is slowed down by salt (A). At high ionic strengths (250 mM KCl) and higher phosphate buffer concentrations (1 mM) the proton release lags slightly behind the formation of the intermediate M-412 and proton reuptake by the purple membrane parallels the slow decay process of M-412 or the decay of O-640.



M-Pos254 PHOTOTACTIC RESPONSES MEDIATED BY SR-I IN *H. HALOBIUM* RECONSTITUTED WITH ALL-*TRANS* RETINAL AND A SERIES OF RING DESMETHYL AND ACYCLIC ANALOGUES. Donald A. McCain[a], L. Amici[a], C.A. Hasselbacher[a], and John L. Spudich[ab]. [a]Dept. of Anatomy and Structural Biology, and [b]Dept. of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx , N.Y., 10461.

A retinal-deficient mutant which contains the SR-I apoprotein was reconstituted with a series of ring desmethyl and acylic analogues of all-*trans* retinal synthesized by Drs. M. Okabe and V.J. Rao in the laboratory of Koji Nakanishi. The analogues increase the $t_{1/2}$ values for thermal decay of the S_{373}-like intermediates in the SR-I photocycle (McCain *et al.* Biophys. J. 49, 478a. 1986). Phototactic responses to reversal-inducing saturating stimuli were determined by computerized motion analysis at 37°C (method of Sundberg *et al.* Biophys. J. 50. 1986). The SR-I photocycle kinetics measured by flash photolysis of whole cells at 37°C, gave $t_{1/2}$ values ranging from 640ms for all-*trans* retinal to 4s for the slowest analogue pigment. These *in vivo* values are considerably larger than those obtained from membrane vesicles and are more directly relevant to the behavioral measurements. The data indicate: (1) The analogues reconstitute both SR-I attractant and SR-I two-photon repellent responses. (2) Cells with $t_{1/2}$ values approximately twice that of the native pigment show similar attractant responses, while cells with SR-I kinetics 3X -6X slower show shifts in the response distributions to longer times. This indicates that the decay of the S_{373} intermediate is one of the rate limiting steps in the SR-I mediated attractant signaling pathway. (3) The flash induced difference spectra did not indicate the presence of intermediates in the analogue SR-I photocycles which were not in the native pigment photocycle that could account for the observed behavioral perturbations, which are explicable solely on the basis of kinetic differences.

M-Pos255 ABRUPT ONSET OF LARGE SCALE NONPROTON ION RELEASE IN PURPLE MEMBRANES CAUSED BY INCREASING pH OR IONIC STRENGTH. T. Marinetti, The Rockefeller University, 1230 York Avenue, New York NY 10021

Nonproton ion movements detected by conductivity changes in suspensions of photo-excited purple membrane (PM) are very sensitive to pH and ionic strength. At pH 7 and low salt (20 mM), a nonproton component to the conductivity transient is seen which corresponds to fast ion uptake followed by release. By either raising the pH above 7.5 or increasing the ionic strength above 100 mM, a dramatic change occurs: the magnitude of the conductivity signal increases sharply and its sense changes. The signal is then dominated by nonproton ion release followed by uptake. These results can be interpreted in terms of light-induced changes in the population of counterions condensed at the PM surface caused by changes in the surface charge density. The critical charge density for ion condensation to occur is evidently achieved near pH 7 by ionizing dissociable membrane groups either by titration (increase pH) or by shifting pK values (increase ionic strength). Such large nonproton ion release then uptake is not seen when the PM is dissolved in Triton X-100 or in phospholipid vesicles. It therefore appears to be associated with the aggregated state of bacteriorhodopsin in the PM.

This work was supported by NIH grant GM32955-02.

M-Pos256 IN VITRO ACTIVITY OF PROTEIN KINASE C IS ATTENUATED IN THE PRESENCE OF 100 mM KCl.
Anita K. Costa and J. R. Trudell, Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

When activated, protein kinase C is known to migrate from the cytosol to the plasma membrane. This activation occurs in the presence of diacylglycerol (DAG), phosphatidylserine (PS) and Ca^{++}, and hence, the enzyme is called phospholipid-Ca^{++}-dependent protein kinase. To date the activity of this enzyme and the effects of certain stimulators (e.g. tumor promotors like phorbolmyristoylacetate) on its activity have been studied in an in vitro system utilizing phosphatidylserine micelles with histone as the phosphate acceptor. As this in vitro test system is far removed from the physiological environment of the kinase in the cell, we attempted to measure the activity in a more representative system. The following phospholipids were tested: PS micelles (50 ug/ml); PS:PC multilammelar vesicles (MLV) 1:4 (w/w) (250 ug/ml) and PS:PC MLV 1:4 (w/w) (50 ug/ml). The PS micelles alone were tested to provide direct comparison to the conventional in vitro test system of Nishizuka et al. All assays were performed in the presence and absence of 5 mol % DAG and in the presence and absence of physiological KCl concentrations. 100 mM KCl decreased the protein kinase C activity in micelles of PS + DAG by 35% and PS - DAG by 63%. In the PS:PC MLV of either 50 or 250 ug/ml, the kinase activity was attenuated by KCl to the same extent in the presence and absence of DAG, viz. 69% and 72%, respectively. Therefore, in both the standard micelle assay and MLVs, the phosphorylating ability of protein kinase C in the presence of KCl is reduced to that of this enzyme in the absence of Ca^{++} and phospholipid, i.e., in its inactivated state. This finding raises a question about the relevance of the assay to cellular physiology.

M-Pos257 COMPARATIVE EFFECTS OF ABA AND IAA ON LIPID BILAYERS.
William Stillwell*, Stephen Wassall+, Daniel Belcher*, Blair Brengle* (Intr. by C. Schauf). Depts. of Biology* and Physics+, IUPUI, Box 647, Indianapolis, IN 46223

The plant hormones abscisic acid (ABA) and indole-3-acetic acid (IAA) are small, monocarboxylic acids of similar pKa's and organic/water solubilities. In living systems however, they support much different functions, presumably due to their association with specific protein receptors. Recent evidence has indicated both hormones may also greatly affect phospholipid bilayers. Here we compare the effects of ABA and IAA on several types of protein-free phospholipid bilayer membranes using a variety of biophysical techniques. By measuring order parameters and correlation times using NMR and ESR techniques, lipid vesicle stability and aggregation, and permeability to $^{3+}$Pr, Cl^- and the non-electrolyte erythritol, we conclude that IAA interacts with the head group component of phosphatidycholine while ABA acts at a part of a mixed component bilayer where there is a discontinuity between different head group types. A clear distinction can be made between the effects of ABA and IAA on lipid bilayers.

M-Pos258 HYPERPOLARIZATION DURING PROLIFERATION OF RAT AORTIC SMOOTH MUSCLE CELLS IN CULTURE.
M.G. Blennerhassett, M.S. Kannan and R.E. Garfield, Neurosciences Department, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada.

The membrane potential (E_m) of aortic smooth muscle cells (SMC) from Sprague-Dawley (SD), Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats was measured during proliferation in culture. These cells show a partial loss of the differentiated state that parallels changes seen in vivo during replication and regeneration. E_m of SMC in primary cultures at 24-48 hours was -29.9 ± 2.9S.D. (64)mV but was -51.1 ± 4.8(130)mV by 3 weeks in vitro. E_m showed similar changes in explant cultures upon subculturing: E_m was -56.1 ± 6.4(58)mV in explant cultures at 3 weeks in vitro, but in a series of parallel subcultures, E_m was -33.1 ± 9.1(21) at 24 hours and gradually hyperpolarized to -58.3 ± 7.1(91)mV by 3 weeks. Since E_m of rat aortic SMC in vivo is -55mV, this suggests that E_m depolarizes upon initiation of mitosis. In cohort cultures initiated at different cell densities, hyperpolarization of E_m was a continuous process during cell proliferation that stabilized when cell number reached a plateau, and was not a time dependent process. Although growth was more rapid in SMC from SHR than from WKY, hyperpolarization of E_m occurred similarly in SMC from SD, WKY and SHR, and it appeared that E_m was a predictable function of the cell density in culture. Therefore, cell interactions during growth in vitro and in vivo may regulate both cell density and the controlled reacquisition of differentiated characteristics. (Supported by the Canadian Heart Foundation).

M-Pos259 CYCLIC AMP/Ca INTERACTION ON THE ELECTRICAL COUPLING IN HEART. Walmor C. De Mello, Department of Pharmacology, Medical Sciences Campus, GPO Box 5067, San Juan, Puerto Rico 00936.

The cyclic AMP hypothesis (De Mello, 1983) was further investigated by studying the influence of intracellular injection of cAMP on the electrical coupling of canine Purkinje cells. It was found: 1) that V_2/V_1 was increased within 30 sec. reaching a maximum in 60-90 sec; 2) the effect of cAMP was completely reversibly; 3) the time constant of cell membrane of the injected cell was reduced from 22.4 to 16 ms. In fibers exposed to dB-cAMP (5 x 10-4 M) plus theophylline (0.4 mM) for 80 min. the space constant was increased by 23.5% while the input resistance was reduced. r_m was slightly decreased while r_i fell by 39%. As cAMP increases g_j within seconds it is concluded that cAMP is enhancing unit channel permeability. In fibers exposed to high Ca (6 mM) solution, however, injection of cAMP caused a rise in V_2/V_1 followed by drastic decrease in V_2/V_1. EGTA (buffered to pH 7.3) injected into the same cell (with double barreled electrode) reversed the decline in V_2/V_1 caused by cAMP. Moreover, the cell decoupling produced by Ca injection was reversed by cAMP injection into the same cell. (Supported by Grants HL-34148 and RR 08102.)

M-Pos260 REGIONAL DISTRIBUTION OF ENZYMES MEDIATING cAMP ACTIONS IN THE MAMMALIAN LENS. K. Hur, and C. Louis. (Intr. by R. Lovrien) Dept. of Vet. Biol., Univ. of Minn., St. Paul.

To define the role of cAMP-dependent protein kinase (cAMP-PK) in the regulation of lens membrane functions, we have examined the regional localization of this enzyme, its substrates, and adenylate cyclase (AC) in the bovine lens. Major substrates (Mr = 28 kDa, 26 kDa, and 18 kDa) for exogenously added cAMP-PK were identified in buffer-washed (BW) lens membranes (in which membrane-bound activities were preserved). Pi incorporation into the 28 kDa + 18 kDa components was approx. 3-fold greater in lens cortex (C) than in lens nucleus (N) membranes; in contrast, Pi incorporation into the 26 kDa component (which comigrated with MP26, the major lens membrane protein) was approx. 4-fold greater in N than in C. When endogenous cAMP, generated by the lens AC in BW membranes, was used to activate exogenously added cAMP-PK, the same lens membrane components were phosphorylated indicating that the lens AC could control the phosphorylation of these membrane substrates *in vivo*. To further examine this possibility, lens AC activators were added to a total lens homogenate that contained lens AC, as well as the lens cAMP-PK and membrane kinase substrates. Only C membrane substrates were phosphorylated, which correlated with the localization of AC to this region of the lens. However, addition of exogenous cAMP to C or N homogenates resulted in significant phosphorylation of both C and N substrates, indicating cAMP-PK is present in both regions of the lens. We conclude that previously identified *in vitro* lens membrane substrates for cAMP-PK can be phosphorylated under conditions approximating the *in vivo* situation. Furthermore, there appears to be a regional localization of the enzymes and substrates mediating cAMP effects in the mammalian lens. Supported by NIH EY-5684.

M-Pos261 LARGE CONDUCTIVITY CHANNELS IN PATCH PIPETTE LIPID BILAYERS FORMED BY LENS JUNCTION PROTEIN (MIP26). J. Wojtczak, S. Girsch*, P. Shrager and C. Peracchia. Department of Physiology, University of Rochester Medical Center, Rochester, NY 14642

Using a spectrophotometric swelling assay we have previously shown that liposome vesicles incorporated with purified lens junction protein (MIP26) are permeable to solutes as large as 1.5 kDa and gated by Ca^{++}-CaM. Electrical properties of these reconstituted lens channels have now been characterized on patch pipette tips following a modified dipping procedure as described by Coronado and Latorre (Biophys. J., 43, 232, 1983). MIP26 was extracted from urea and caustic-washed bovine lens with $CHCl_3$:MeOH/3:1, taken up in hexane and mixed with PC:Chl/1:1, also in hexane. A monolayer of lipid-protein was formed at the air/water interface of a saline solution (0.145 M NaCl, Ca^{++} buffered to 10^{-7} M, pH 7.4). Bilayers of 2-10 GΩ were formed at the tip of the patch pipette by withdrawing and reimmersing the pipette. Ionic currents could be recorded both at negative and positive voltages under voltage clamp reflecting discrete channels. Two types of channels were observed. Large channels with a conductance of approximately 104 pS, exhibited long open times, flicker-like activity, and short closed times. The conductance of these channels is consistent with that of single gap junction channels recorded in cell pairs. Smaller channels with a conductance of 18-25 pS, exhibited short open times and a lack of flicker-like activity. They may represent subconductance states, new subunit or conformational states or contaminant. Since channel activity ceases spontaneously within 1 hr, experiments with anti-MIP26 are as yet inconclusive. Similar channels have been observed using purified rat liver gap junction protein. Supported by NIH grants GM 20113 to CP and EY 06467 to SG.

M-Pos262 ION ACCUMULATION AND DEPLETION NEAR GAP JUNCTION CHANNELS. P. R. Brink, S. W. Jaslove, R. Mathias, G. Baldo Departments of Anatomical Sciences and Physiology and Biophysics, S.U.N.Y. at Stony Brook, L.I., N.Y. 11794

Gap junction channels of earthworm septal junction have been shown by the voltage clamp method to be electrically linear (Verselis and Brink, B.J. 45:145, 1984). However, long duration voltage steps induce a slow junctional current relaxation (on the order of seconds) to about 85% of its initial value. We have considered three possible models to account for this relaxation: (1) incomplete voltage dependent gating, (2) current dependent reduction in conductance due to slowly permeating ions, (3) local diffusion potentials due ion accumulation and depletion at the junctional interfaces. Experiments support the third hypothesis: when returned to a transjunctional potential of zero following a step of either sign, a transient current of opposite sign is induced; the first two models predict zero current for zero driving force. Also, when returned to a potential of equal amplitude but opposite sign, the amplitude of the current relaxation is twice that of the initial relaxation. Lastly, experiments utilizing small voltage steps indicate no conductance change occurs during the relaxation. These data suggest that the observed current relaxation is due to differential mobility of cations and anions through gap junction channels which results in a local Nernst potential across the channels in opposition to the applied voltage step.

Supported by NIH grants HD 31299, HL 36075, EY06391.

M-Pos263 A DIFFUSION MODEL FOR A MULTICELLULAR SHEET: THE INFLUENCE OF GAP JUNCTIONS ON THE RATE OF DIFFUSION. By. B. Bunch, S. Ramanan and P. R. Brink. Department of Anatomical Science, SUNY at Stony Brook, Stony Brook, NY.Y. 11794.

A Fickian diffusion model has been developed for a monolayer of cells where all the cells are linked by gap junctions on their lateral surfaces. Each cell could potentially be connected to 2, 4 or 6 other cells and the gap junctional area of contact must be the same of all interfaces. Cell shape must also be uniform, that is 4 sided or 6. The junctions are assumed to lie perpendicular to the plane of the cell sheet.

The model predicts that when the junctional membrane permeability (P_j is in the range of $1X10^{-3}$ cm/s or has a junctional membrane diffusion coefficient (D_j) of $1.5X10^{-9}$ cm^2/s ($P_jX1.5nm$ where 1.5nm equals the junctional membrane width) that the cytoplasmic diffusion coefficient will dominate the diffusion profile with time. Most small solutes have cytoplasmic diffusion coefficients in th range of $5\text{-}1X10^{-7}$ cm^2/s. Thus when the ratio of the cytoplasmic diffusion coefficient to the junctional membrane diffusion coefficient (D_c/D_j) is in the range of 400, diffusion through the cytosol is the rate limiting step for solute translation mobility. As D_c/D_j increases (4000 or greater) it is the junctional membrane channels which are the major determinants of the lateral spread of solutes through a tissue. This prediction has implications for the role junctions play in determining the true syncytial nature of tissues. The mobility of the solute in the cytoplasm may turn out to be a more important determinant of solute spread than junctional membrane patency in embryonic tissues, as an example, where temperal changes in morphorgens is considered to be the underlying mechanism of differentiation. This work is supported by NIH Grant GM 24905.

M-Pos264 PHOSPHOLIPID AND CHOLESTEROL DEPENDENCE OF THE (Na^+ + Mg^{2+})-ATPase FROM ACHOLEPLASMA LAIDLAWII B MEMBRANES. R. George, R.N.A.H. Lewis and R.N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The (Na^+ + Mg^{2+})-ATPase purified from A. laidlawii B membranes was reconstituted with a number of different phospholipids of varying fatty acid chain length and degree of unsaturation using a detergent solubilization and dialysis procedure. The successful reconstitution of the enzyme was accompanied by an enhancement of its specific activity. The activity of the ATPase in the reconstituted complex was determined at various temperatures so as to change the fluidity and phase state of the phospholipids. At comparable temperatures the enzyme activity is dependent on lipid phase state and is considerably higher in the presence of liquid-crystalline than in gel-state phospholipid. Phosphatidylcholines (PCs) with fatty acid chain lengths of 14-18 carbons and varying degrees of unsaturation successfully reconstitute the enzyme. However, the shorter-chain ditridecanoyl PC does not support ATPase activity. Among the various phospholipids tested, PC and phosphatidylethanolamine effectively reconstitute the enzyme, whereas phosphatidylserine (PS) and phosphatidylglycerol (PG) are ineffective. PS and PG show an inhibitory effect on the ATPase activity when reconstituted in the presence of dimyristoyl PC. These results suggest that, contrary to earlier reports, anionic phospholipids may not be an absolute requirement for the function of this ATPase. We also show that the presence of cholesterol in the reconstituted phospholipid-ATPase vesicles has little effect on enzyme activity above the phase transition temperature of the phospholipid but increases activity below this temperature, probably by disordering the gel state of the phospholipid bilayer.
(Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.)

M-Pos265 FRACTIONATION AND RECONSTITUTION OF CARDIAC SARCOLEMMAL PROTEINS AND Na-Ca EXCHANGE BY HPLC. Steven Kleiboeker, James Carlton, Michael Rovetto, and Calvin C. Hale, Departments of Veterinary Biomedical Sciences and Medical Physiology, Dalton Research Center, University of Missouri-Columbia, Columbia, MO 65211

Previous studies showed that cardiac sarcolemmal (SL) vesicle proteins could be extracted, fractionated by standard gel permeation column chromatography, and reconstituted into proteoliposomes (PNAS 81:65-69, 1984). Unfortunately, this procedure resulted in a significant loss (93%) of Na-Ca exchange activity in a time dependent manner. In the present study, we have utilized gel permeation by HPLC to fractionate cardiac SL proteins prior to reconstitution. SL vesicle proteins from bovine ventricular tissue were extracted in 2% Na cholate, 100 mM NaP, pH 7.3, 25 mg/ml soy bean phospholipids (asolectin). Extracts were injected onto a BioGel TSK 40XL or 30XL (BioRad) connected to a Perkin-Elmer Series 4 HPLC pre-equilibrated with 2% Na cholate, 100 mM NaP, pH 7.3, 2.5 mg/ml asolectin. Eluted fractions were mixed 1:1 with equilibration buffer containing 50 mg/ml asolectin. Proteoliposomes containing fractionated SL proteins were formed by detergent dilution followed by two washes in 160 mM NaCl, 20 mM MOPS/TRIS pH 7.4. Column fractions were assayed by absorbance at 280 nm, protein content, SDS-PAGE, and Na-Ca exchange activity. Na-Ca exchange activity was present in the column void and in a small shoulder that preceded a major peak containing proteins smaller than 68 KDa. Recovery of total protein and activity on the TSK 40XL column was 91±7.4% and 68± 11.3% (n=4) respectively. The results suggest that protein(s) responsible for catalyzing the exchange of Na+ for Ca2+ in SL vesicles is larger than 68 KDa. HPLC offers a convenient method for quantitative analysis, preparative isolation, and reconstitution of fractionated SL proteins, including recovery of Na-Ca exchange, while avoiding a significant loss in transport activity.

M-Pos266 **ACETYLCHOLINE RECEPTOR IN PLANAR POLYMERIZED BILAYERS--TOWARD A RECEPTOR-BASED BIOSENSOR**

Paul Yager[1], Adam W. Dalziel[2], Jacque Georger[3], Ronald R. Price[1] and Alok Singh[3]

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We are developing an acetylcholine receptor-based biosensor by combining current techniques in electrophysiology with newly developed methods of forming polymerized membranes. To produce the sensing element of the biosensor we require a stable planar lipid bilayer support for the receptor. We are developing such supports by combining polymerized phospholipid monolayers with interfacial layers formed from vesicles containing acetylcholine receptor.

Interfacial layers were formed by applying lipid vesicles containing the acetylcholine receptor to an air-buffer interface. The transferred interfacial layers contained vesicle-like structures and small isolated particles, of dimensions similar to those of acetylcholine receptor, interspersed in the film. Transferred monolayers of a polymerized diacetylenic phosphatidylcholine exhibited a remarkable degree of mechanical stability. Electron microscopic examination of the polymerized monolayers indicated a high degree of integrity. The interfacial layer formed from acetylcholine receptor vesicles was effectively transferred to a polymerized phospholipid monolayer. The method of Coronado and Latorre (Biophys. J. 43:231-326 (1983)) has also been used to assemble asymmetric bilayers of monomeric and polymerized lipids in a configuration that allows electrical measurements to be made. Patch clamp measurements of such bilayers will be reported.

M-Pos267 CALCIUM SPECIFIC CHANNELS INDUCED BY AGGREGATION OF THE Fc_ϵ RECEPTOR FROM MAST CELLS. A.Corcia, B.Rivnay, and I.Pecht. Depts. of Membrane Research and Chemical Immunology. The Weizmann Institute of Science, Rehovot, Israel 76100.

Mast cells and basophiles release histamine and serotonine in response to aggregation of their Fc_ϵ receptors ($Fc_\epsilon R$). The release occurs only in the presence of extracellular calcium and requires a transient rise in cytosolic free calcium. We have previously shown that ion channel activity can be induced by aggregation of $Fc_\epsilon R$ in lipid bilayers containing two protein complexes isolated from membranes of rat basophilic leukemia cells (Line RBL-2H3). These components are the $Fc_\epsilon R$ complex itself and the cromolyn binding protein (CBP). The ion specificity of these channels has been studied by examining the conductance and open time characteristics of antigen-induced channel events in micropipette-supported bilayers containing both protein components. When the bilayer is exposed to solutions containing 150 mM of either NaCl or KCl and a gradient of calcium is present (1.8 mM $CaCl_2$ in the bath, 0.1μM $CaCl_2$ in the pipette), two populations of antigen-induced channels are observed. One, accounting for 90% of all the events, has a conductance of 3 pS and is calcium-specific (reversal potential of 130 mV, practically equal to the Nernst potential for calcium in these experimental conditions). The second population, 5-10% of the events, has a higher conductance (20 pS) and allows the passage of both calcium and sodium (reversal potential 16 mV). Both types of events have short open times (in the range 1-5 ms), appear mainly isolated and with relatively long periods of non-conducting state in between. Thus, antigen-induced calcium-specific channels are activated in this reconstituted system. Strontium can substitute for calcium as the current carrier through these channels, although with slightly different channel characteristics.

M-Pos268 SPECTROSCOPIC STUDIES OF BACTERIOPHAGE LAMBDA MEDIATED DNA INJECTION INTO RECONSTITUTED LamB PHOSPHOLIPID VESICLES. Steven L. Novick and John D. Baldeschwieler, Division of Chemistry and Chemical Engineering, Mail Code 127-72, California Institute of Technology, Pasadena, CA 91125.

LamB is an integral outer membrane maltose transport protein of E. coli which also serves as the bacteriophage lambda receptor. Phage inactivation by LamB has typically been quantitated by inhibition of placque formation on a plated indicator strain. The assay is indicative only of phage binding and not DNA injection, since binding inactivation occurs whether or not it is followed by DNA injection. The goal of these studies is to develop quantitative physical assays for the injection process.

The assays are based on encapsulation of DNA-sensitive reporter molecules. By encapsulating chelated ^{111}In, gamma ray perturbed angular correlation spectroscopy is used as a steady state probe of the rotational mobility of the ^{111}In nucleus as a function of binding to injected DNA. Kinetics of injection are assayed by enhancement of encapsulated ethidium bromide fluorescence upon binding to injected DNA.

These data are consistent with that published by Roessner et. al. [J. Biol. Chem. 258 643 (1983)] and help to give a more detailed picture of binding and injection at a molecular level.

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M-Pos269 CHANNELS MODULATED BY CYTOSKELETAL PROTEINS, P. Vassilev, H.T. Tien, and M. Kanazirska, Biophysics Laboratory, Department of Physiology (Giltner Hall), Michigan State University, East Lansing, MI 48824

Cytoskeletal components were found to influence various membrane processes, including ion transport and excitability. There is evidence that calmodulin antagonists affect Ca^{2+}-transport events and that microtubule proteins modify excitation mechanisms. Ca^{2+}/calmodulin-dependent protein kinases from brain which can interact with microtubule-associated proteins were found to modulate inactivation of ionic currents. In an attempt to clarify the interrelationships between cytoskeleton and Ca^{2+} transport we studied the effects of the anticalmodulin agent trifluoperazine and of microtubule proteins on the Ca^{2+} channel activity in patch clamp bilayers containing lipid and proteolipid components from brain microsomal membranes. We observed drastic decrease in the mean open time of the channels in the presence of 20-40 μM trifluoperazine. The channel activity was substantially higher in the presence of microtubule proteins. A dramatic increase in the stimulatory effect of guanosine 5'-triphosphate (GTP) was observed in this case. GTP (50 μM) induced a two-fold increase in the open state probability in the presence of microtubule proteins. Other nucleotides (ATP, ITP) did not exert any significant effects on the channel activity when used at the same concentrations. However, a substantially increased open state probability was observed under the influence of inositol trisphosphate (25 μM), which is known as an agent exhibiting its effects on intracellular Ca^{2+} homeostasis by mechanisms involving Ca^{2+}-dependent protein kinases.

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M-Pos270 EFFECT OF MONOMER SIZE ON LIPOPOLYSACCHARIDE INTERACTIONS WITH CELLS OR MODEL MEMBRANES. Hung-Yueh Yeh, Ray M. Price and Diane M. Jacobs, Dept. Microbiology, SUNY-Buffalo, Buffalo, NY 14214.

Lipopolysaccharide (LPS), a major component of the Gram-negative bacterial outer membrane, consists of three structural regions: Lipid A, core oligosaccharide and O-antigen repeating side chain, in that order. LPS has various biological effects that depend on the presence of Lipid A. However, the effect of the other structural regions on LPS activities has not been well studied. In aqueous solution, LPS exists as an aggregate of a mixture of LPS monomers differing in the number O-antigen repeating units added to the core structure. The LPS (from Salmonella typhimurium, ATCC 14028) aggregate can be separated into three fractions, based on the number of repeating units, on a Sephadex G-200 column in the presence of sodium deoxycholate: in order of elution, fraction 1 with an average of 70 repeating units; fraction 2, 30 repeating units; and fraction 3, 2 repeating units. We have modeled the initial interaction of LPS with cell membranes as consisting of two steps: adherence and coalescence. In the biological assay of LPS-induced murine splenocyte mitogenesis, the optimal concentrations of fractions 1, 2 and 3 were 1.10 nmol/ml, 0.78 nmol/ml and 0.71 nmol/ml, respectively. In the biophysical assay of LPS-induced changes in diphenylhexatriene anisotropy in small unilamellar vesicles, there was no difference, on a molar basis, between the three fractions. These results suggest that the length of the O-antigen repeating units modulate LPS activities. Monomer length may affect either the adherence step or some other interaction between LPS and a cell surface structure involved in signal transduction.

M-Pos271 WHAT ARE MIXED MICELLES?: THE COMPOSITION OF EGG PC-OCTYLGLUCOSIDE MIXED MICELLES. Ofer Eidelman, Anne Walter and Robert Blumenthal, Section on Membrane Structure and Function, LMMB, NCI, NIH, Bethesda, MD.

The molecular composition of octylglucoside-PC (OG-PC) mixed micelles, and its dependence on detergent and lipid concentrations, were studied by measuring fluorescence resonance energy transfer between trace amounts of the fluorescent lipid probes NBD-PE (donor) and Rho-PE (acceptor), which were incorporated into PC SUV. These were dissolved by slow addition of OG while monitoring NBD fluorescence. An expression was derived for the average number of lipids per micelle (<n>) assuming a Poissonian distribution of lipids among micelles and complete fluorescence quenching when both Rho-PE and NBD-PE are found in the same micelle. According to this derivation, <n> can be determined from the observed donor fluorescence for a given mol fraction acceptor. By varying the acceptor concentration, and observing the region where calculated <n> values coincided, we determined the limitations on the validity of the assumptions.

The composition of mixed micelles changed as a function of lipid and detergent concentrations: (1) The total concentration of detergent needed to observe micelles with any given <n> was linearly dependent on [lipid]. (2) <n> was 1 at and above the cmc of OG, and it increased exponentially as [detergent] decreased toward the mixed-micelle-to-disc phase boundary. (3) The average number of detergent molecules per mixed micelle increased with <n>. (4) The OG-PC micelles at optical clarity (solubilization) contained 40-50 PC molecules. This large number may explain why OG is often able to conserve membrane protein function during the solubilization procedure. These results have implications for designing membrane reconstitution protocols.

M-Pos272 THEORY OF PASSIVE PROTON/HYDROXYL CONDUCTANCE ACROSS LIPID BILAYERS. John F. Nagle. Departments of Physics and Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213.

The large measured proton/hydroxyl permeability at pH 7 suggests a special transport mechanism. The nearly constant conductance from pH 2 to 11 rules out most models, such as the weak acid carrier model. The suggestion of Nichols and Deamer that transient hydrogen bonded chains (HBC) of water might provide the special mechanism is investigated theoretically. Three distinct models that all employ HBCs are consistent with the constant conductance with varying pH experimental result. In model A the turning defect, which is independent of pH, is rate limiting. In model B the HBC disintegrates with attempted passage of the turning defect and the rate limiting step is the formation of chains. In model C (proposed by W. Nichols) two half chains with opposite charges meet with charge recombination effecting transport, with the recombination step, independent of pH, being the rate limiting step. However, kinetic calculations show that the three models predict different current characteristics with increasing pH gradients and electrical potentials. These differences may be useful in determining which is the appropriate model for lipid bilayers.

M-Pos273 PROTON/HYDROXIDE CONDUCTANCE AND PERMEABILITY THROUGH PHOSPHOLIPID BILAYER MEMBRANES: EFFECTS OF LONG-CHAIN FATTY ACIDS. John Gutknecht, Physiology Dept., Duke University, and Duke Marine Lab., Beaufort, NC 28516.

Mechanisms of proton/hydroxide conductance ($G_{H/OH}$) were investigated in planar (Mueller-Rudin) bilayer membranes made from decane solutions of phospholipids or phospholipids plus phytanic acid (a 20-carbon, branched chain fatty acid). At neutral pH, membranes made from diphytanoyl phosphatidylcholine or bacterial phosphatidylethanolamine had $G_{H/OH}$'s in the range of $(2 - 5)_{-1} \times 10^{-8}$ S cm^{-2}, corresponding to H^+/OH^- "net" permeabilities of about (0.4 - 1.0) x 10^{-5} cm s^{-1}. $G_{H/OH}$ was inhibited by serum albumin, phloretin, glycerol and low pH, but was increased by chlorodecane and voltages > 80 mV. Water permeability and $G_{H/OH}$ were not correlated, suggesting that water and H^+/OH^- cross the membrane by separate pathways. Addition of phytanic acid (2 - 20 mol%) to the phospholipids caused an increase in $G_{H/OH}$ which was proportional to the first power of the phytanic acid concentration. In phytanic-acid containing membranes, $G_{H/OH}$ was inhibited by albumin, phloretin, glycerol and low pH, but was increased by chlorodecane and voltages > 80 mV. The pH dependence and concentration dependence suggest that phytanic acid acts as a simple (A^- type) proton carrier. The similarities between the behavior of $G_{H/OH}$ in unmodified and phytanic-acid containing membranes suggests that phospholipids may contain weakly acidic contaminants which cause most of $G_{H/OH}$ at pH > 4. The ability of phytanic acid to act as a weak protonophore may help to explain the toxicity of phytanic acid in Refsum's disease, a metabolic disorder in which phytanic acid accumulates to high levels in plasma, cells and tissues. (Supported by NIH grant GM 28844.)

M-Pos274 CHARACTERIZATION OF Na-K-Cl COTRANSPORT IN CULTURED HT-29 HUMAN COLON CARCINOMA CELLS. H.D. KIM AND J.T. TURNER, DEPT. OF PHARMACOLOGY, UNIV. OF MISSOURI, COLUMBIA, MO 65212

The presence of a Na-K-Cl-cotransport pathway has been demonstrated in the HT-29 human colon carcinoma cell line. Ouabain resistant-bumetanide sensitive (OR-BS) ^{86}Rb influx in attached HT-29 cells is about 14.18 ± 1.19 nmol/mg x min at 25°C in incubation media containing (mM); 5 K, 126 Na, 131 Cl, 5 glucose, 1 sodium phosphate, 10 tris-Hepes pH 7.4, and 0.2 ouabain either with or without 0.1 bumetanide. The identity of this pathway as a Na-K-Cl cotransporter has been deduced from the following findings: A) OR-BS ^{86}Rb influx ceases if the external Cl, $[Cl]_o$, is replaced by NO_3 or $[Na]_o$ by choline; B) OR-BS ^{86}Rb influx increases progressively as $[Cl]_o$ is raised, whereas the $[Na]_o$ dependency reaches a saturation near 10-15 mM $[Na]_o$; and C) concomitant measurements of ^{86}Rb, ^{22}Na, and ^{36}Cl influxes indicate that the stoichiometry of the cotransport system appears to approach a ratio of 1Na:1K:2Cl. In addition, OR-BS ^{86}Rb influx is exquisitely sensitive to cellular ATP levels. We found that deoxyglucose causes a rapid ATP loss. A depletion of the normal ATP content of 35-40 nmol/mg protein to 10-15 nmol/mg protein, a concentration at which ouabain-sensitive ^{86}Rb influx is unaffected, completely abolishes ^{86}Rb cotransport. Changes in cell volume, either shrinkage or swelling, do not influence OR-BS ^{86}Rb influx. Unlike the dependence on $[Na]_o$ or $[Cl]_o$, OR-BS ^{86}Rb influx in response to a progressive increase in $[K]_o$ exhibits a well defined maximum at 5 mM, followed by a virtual arrest of the transport taking place near 25 mM $[K]_o$. Since Ba^{++} reduces the ^{86}Rb cotransport, it is suggested that the Na-K-Cl cotransport pathway is intimately associated with a K-channel.

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M-Pos275 **LOW DENSITY LIPOPROTEIN CHOLESTERYL ESTER HYDROLYSIS IN NORMAL AND WOLMANS DISEASE FIBROBLASTS: USE OF A SPECIFIC FLUORESCENT LABEL TO FOLLOW INTRACELLULAR LIPID TRAFFICKING.** Martino Picardo, Eliot Sugarman, and Henry J. Pownall, Baylor College of Medicine, Houston, Texas 77030

Both DPH-fluorescent and ^{14}C-radiolabeled cholesteryl ester analogs were incorporated into LDL and their uptake and intracellular localization investigated in normal human fibroblasts and those derived from patients with Wolmans disease, in which lysosomal cholesteryl esterase activity is absent. In normal fibroblasts, both radioactive and fluorescent labels were discernible within lysosomal, plasma membrane, and golgi-enriched fractions. Moreover, chemical analysis showed that portions of both labels had been converted to free fatty acid and derivatives of polar and nonpolar glycerides. The highest proportion of labeled products was found in fractions enriched with plasma membranes, followed by golgi particles and lysosomes. By contrast, in the fibroblasts from Wolmans subjects, neither the radioactive nor the fluorescent cholesteryl esters were degraded and the labels were found exclusively within the lysosomal fractions. A similar result was observed in normal fibroblasts when the labeled cholesteryl esters in LDL were replaced by their radiolabeled ether analogs. We confirm the reports that free fatty acids derived from LDL cholesteryl esters in normal fibroblasts are precursors for synthesis of neutral and phosphoglycerides; in Wolmans fibroblasts, the cholesteryl esters accumulate in lysosomes and are not precursors of other lipids. We conclude that cholesteryl ethers in normal fibroblasts appear to be good cellular models of Wolmans disease. These data demonstrate that a DPH-labeled lipid, which is highly fluorescent, has biochemical properties and behavior similar to that of its natural analog.

M-Pos276 PURIFICATION OF BRUSH BORDER MEMBRANE VESICLES FROM PIG SMALL INTESTINE BY FILTRATION ACROSS GLASS FIBERS. Chenu C., Watkins D., Gobin R., Bourguet J. and Ripoche P., Department of Biology, CEN Saclay, 91191 France.

Brush border vesicles prepared according to Kessler were suspended in a medium containing 50 mM mannitol, 10 mM Hepes/Tris, pH 7.4, at a concentration of 0.1 mg protein/ml. Increasing volumes of suspension were filtered through Whatman GF/F filters. Up to 10 ml of filtered volume (1 mg protein), the percentage of vesicles retained by the filter was constant, about 25 %. Original vesicles and filtered vesicles were compared in term of protein content, shape, enzymatic activity and transport capacity. Analysis by SDS polyacrylamide (9 %) gel electrophoresis did not show a significant difference in the protein pattern between the two vesicle populations. The specific activity of leucine aminopeptidase, a brush border membrane-bound enzyme, was increased 50 % in the filtered vesicles (from 1.6 to 2.4 µmoles/mg protein/min at 37 °C) with only a moderate loss of yield. Freeze fracture observations indicated that filtration eliminated the large vesicles and left a homogeneous population of vesicles with a mean diameter of 120 nm. Finally the glucose transport capacity of filtered vesicles was superior to that of unfiltered vesicles. Filtered vesicles accumulated glucose up to 60 times compared to 40 times for unfiltered vesicles when incubated in a medium of 0.1 mM glucose, in the presence of an inwardly directed 100 mM NaCl gradient and a potassium-valinomycin generated membrane potential. Preparation of vesicles by filtration allows the production of a homogeneous population of vesicles with a reduced level of contaminants and parasite compartments, suitable for influx and efflux measurements.

M-Pos277 COMPLEXATION AND DISSOCIATION KINETICS OF IONOPHORE A23187 WITH NI^{2+} IN 80% METHANOL-WATER. T.P. Thomas[1], D.R. Pfeiffer[2], and R.W. Taylor[1]. [1]Dept. of Chemistry, Univ. of Oklahoma, Norman, OK 73019 and [2]The Hormel Inst., Univ. of Minnesota, Austin, MN 55912.

Ionophore A23187, A, forms 1:1 and 1:2 metal ion:ligand complexes with Ni^{2+} in 80% methanol-water solvent. Using the stopped-flow technique, we have studied the kinetics of the formation and dissociation reactions of these complexes in 80% MeOH-H_2O at 25.0°C. The formation kinetics were studied as a function of [A], $[Ni^{2+}]$ and pH* and found to obey the rate law: $d[NiA]/dt = (k_A[A^-] + k_{HA}[HA])[Ni^{2+}]$, where k_A and k_{HA} are the rate constants for reactions with the anionic and free acid forms of the ionophore, respectively. Values of k_A and k_{HA} are 1.5×10^6 $M^{-1}s^{-1}$ and $\leq 5\times10^2$ $M^{-1}s^{-1}$, respectively. The kinetics of the dissociation of NiA^+ and NiA_2, induced by mixing with an excess of $HClO_4$, were carried out as a function of $[Ni^{2+}]$ and $[H^+]$. The reaction rates were independent of $[Ni^{2+}]$ and displayed saturation type kinetics with respect to $[H^+]$. The $[H^+]$ dependence of the observed rate constant was of the form; $k_{obs} = (k_d+k_HK_{NIAH}[H^+])/(1+K_{NIAH}[H^+])$, where k_d and k_H are the rate constants for the uncatalyzed and proton-catalyzed dissociation pathways, respectively. K_{NIAH} is the equilibrium constant for $NiA^+ + H^+ \rightleftarrows NiAH^{2+}$; which is rapid relative to the dissociation of $NiAH^{2+}$. The values obtained for k_d, k_H and k_{NIAH} are 0.046 s^{-1}, 0.40 $M^{-1}s^{-1}$ and 690 M^{-1}, respectively. Dissociation of NiA_2 to NiA^+ was too fast for the stopped-flow technique. The kinetics of the ligand exchange reaction, NiA_2+EDTA→Ni(EDTA)+2A^-, were studied as a function of $(EDTA)_{TOT}$ and pH*. The values of the observed rate constant were independent of pH* and $(EDTA)_{TOT}$ and were essentially the same (0.038 s^{-1}) as that obtained for k_d from studies with excess acid. (Supported by NIH grant GM 24701).

M-Pos278 COMPARISON OF E. COLI PROTEIN-DNA INTERACTIONS IN VITRO AND IN VIVO. S. Cayley, B. Richey, M. Mossing, C. Kolka, M.T. Record, Jr., Departments of Chemistry and Biochemistry, University of Wisconsin, Madison WI 53706.

The osmotic adaptability of E. coli involves dramatic changes in its intracellular ionic content. In minimal media, K^+ and Glu^- are the primary osmolytes accumulated by E. coli when external osmolarity rises. Cytoplasmic K^+ and Glu^- levels increase linearly with external osmolarity from 0.22 M and 0.03 M in minimal media to 0.95 M and 0.25 M, respectively, in media with 0.5 M NaCl. In vitro, the sensitivity of the rate of forming transcriptionally active RNA polymerase-promoter complexes to changes in KCl concentrations is typically steep and promoter-specific (1). Small changes in in vitro levels of KCl can therefore markedly alter the relative strengths of different promoters. To test whether the in vivo range of K^+ concentrations alters relative promoter strength in E. coli, levels of β-galactosidase of cells containing the lac Z gene fused to promoters (P_R and P_{RM}) with different in vitro salt sensitivities of interaction with RNA polymerase were compared as a function of external osmolarity. In contrast to the observed in vitro behavior, relative promoter strength does not change as intracellular K^+ content changes. The relative in vivo levels of lac repressor binding to two mutant operators, exhibiting different in vitro salt dependences of repressor-operator affinity (2), are also unaffected by changes in external osmolarity. This paradox remains unresolved, although replacement of KCl by KGlu enhances the in vitro interaction of a wide variety of proteins with DNA at otherwise inhibitory concentrations of K^+.

(1) Roe, J.H. and Record, M.T. Jr., Biochemistry (1985) 24, 4721-4726. (2) Mossing, M. and Record, M. T., Jr. J.Mol. Biol. (1985) 186, 295-305.

M-Pos279 REPLACEMENT OF KCl BY KGLUTAMATE DRAMATICALLY ENHANCES PROTEIN-DNA INTERACTIONS IN VITRO C. Harrison, S. Leirmo, D.S. Cayley and M.T. Record, Jr. (Intro. by Paul Kaesberg), Departments of Chemistry and Biochemistry, University of Wisconsin, Madison, Wisconsin 53706.

Protein-nucleic acid interactions and many other noncovalent interactions of biopolymers in vitro are highly sensitive to both ion concentration and type. E. coli however, grows over a wide range of intracellular ionic conditions and utilizes ions as intracellular osmolytes. The primary osmolytes accumulated by E. coli are K^+ and glutamate (Glu^-), while the intracellular concentration of Cl^- is very low. Since Glu^-, and not Cl^-, appears to be the major anion in many types of cells, we have investigated the effects of the substitution of Glu^- for Cl^- on several protein-nucleic acid interactions in vitro. Kinetics of association of RNA polymerase with promoters were measured under pseudo-first order conditions of polymerase excess in varying concentrations of either KCl or KGlu at 37°. The substitution of Glu^- for Cl^- increases the second order rate constant (k_a) by at least 30-fold for λP_R and 10-fold for $\lambda P_R'$ at a given K^+ concentration. For both promoters, substitution of Glu^- for Cl^- allows open complex formation to occur at high K^+ concentrations which inhibit binding in Cl^-. The large cation dependence of k_a and the general features of the binding mechanism are maintained for both promoters when Cl^- is replaced by Glu^-. Similar effects are observed for the restriction enzymes examined, in that the range of salt concentrations over which site-specific DNA cleavage occurs is shifted to higher K^+ concentrations when Cl^- is replaced by Glu^-. These effects of Glu^- may partially account for the apparent disparity observed between typical ranges of salt concentration in vivo and in vitro.

M-Pos280 **SUBUNIT EXCHANGE KINETICS OF A GENE REGULATORY PROTEIN: IMPLICATIONS FOR SPECIFIC COMPLEX FORMATION.** Abraham M. Brown and Donald M. Crothers, Department of Chemistry, Yale University, New Haven, CT. 06510 (Intr. by Gary Brudvig)

A novel method for measuring hetero-subunit association/dissociation of proteins is described. The method is general and works under equilibrium conditions. Biotinylated and radiolabeled preparations of the same protein are mixed under varying solution conditions. After incubation, the mixture is bound to Streptavidin-agarose. The extent of subunit exchange is quantitated by comparing the ratio of bound to unbound radiolabel. Radiolabeled subunits bind to the affinity resin only if they are tightly associated with biotinylated subunits, thus retention of counts due to mixed dimers is indicative of subunit exchange within a protein molecule. This method has been applied to study the exchange behavior of Catabolite Activator Protein (CAP) in the presence of various ligands. Cyclic AMP or specific operator binding reduce exchange kinetics, implying a stabilization of dimer contacts. Under low salt conditions (20mM KPO_4, 1mM EDTA) addition of sonicated DNA gives rise to a dramatic (~50-fold) increase in the exchange rate. This acceleration is DNA concentration dependent and diminishes with increasing ionic strength. The effects of pH and RNA Polymerase have also been examined.

M-Pos281 A SINGLE-STRANDED REGION ON OTHERWISE DUPLEX DNA PERMITS EXTENSIVE BINDING OF REC A PROTEIN TO THE DUPLEX PORTION OF THE MOLECULE. Sandra L. Shaner, John Flory and Charles M. Radding Department of Human Genetics, Yale University, New Haven, CT 06510.

A short single-stranded tail on one end of an otherwise duplex DNA molecule enables recA protein, in the presence of ATP and $MgCl_2$, to form a complex with the DNA which extends into the duplex portion of the molecule. Nuclease protection studies at a concentration of $MgCl_2$ which permits homologous pairing showed that cleavage by restriction endonucleases at sites throughout the duplex region is strongly inhibited, whereas digestion by DNase I is not affected. Local mobility of the recA protein associated with ATP hydrolysis permits the rapid nonspecific cleavage by DNase I while blocking site-specific cleavage. Protection against site-specific cleavage is greater for 5' tailed duplexes than for 3' tailed duplexes for all recA protein concentrations examined. Visualization by electron microscopy of complexes formed at 2mM $MgCl_2$ corroborates the findings of the enzyme protection experiments. The recA protein extends further from the single-stranded tail into the duplex region with time during ATP hydrolysis. Protein complexes on duplexes with 5' tails show a faster rate of extension into the duplex region than complexes with 3' tails. However, the protein tracts on the 3' tailed duplexes are less segmented by regions of free DNA. These results will be discussed with respect to a proposed mechanism for the ATPase activity of recA protein with tailed duplex DNA cofactors.

M-Pos282 SELF-ASSEMBLY OF RECA PROTEIN STUDIED BY ANALYTICAL ULTRACENTRIFUGATION. Stephen L. Brenner, Central Research and Development Department, Experimental Station, E. I. DuPont de Nemours and Co., Inc., Wilmington, DE 19898, and Walter F. Stafford III, Boston Biomedical Research Institute, Boston, MA 02114.

In the initial step of homologous recombination in E. coli, recA protein assembles cooperatively onto ssDNA to form spiral protein helices. The protein also aggregates in solution in the absence of DNA but little is known about the self-assembly process. Since quantitative analysis of recA binding to DNA requires knowledge of the state of assembly of recA protein in solution, we have used sedimentation equilibrium to analyze the assembly process. Over a range of initial loading concentrations and rotor speeds (in either 10 mM Tris-acetate, 10% glycerol pH 7.5, or 50 mM Na-citrate, 5 mM ADP, 5% glycerol, pH 6.0, at 4°C) we find a distribution of oligomers. Under no conditions were detectable amounts of monomeric recA observed. The smallest species detected was a dimer with a molecular weight of 76 kDa. Oligomers containing 9-12 recA monomers were observed at both pH's with no significant amount of intermediate sized polymers. Preliminary analysis suggests that the dimer and the higher molecular weight species are in equilibrium.

M-Pos283 E. COLI SSB PROTEIN FORMS MULTIPLE, DISTINCT COMPLEXES WITH SINGLE STRANDED DNA. W. Bujalowski, T.-F. Wei and T.M. Lohman, (Intr. by E.G. Sander) Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843. We have identified four distinct binding modes for the interaction of E. coli Single Strand Binding (SSB) protein with single stranded (s.s.) DNA based on quantitative titrations which monitor the quenching of the SSB protein fluorescence upon binding to poly(dT) over a range of $MgCl_2$ and NaCl concentrations at 25°C and 37°C. This is the first observation of multiple binding modes for a single protein binding to DNA. These results extend previous studies performed in NaCl (25°C, pH 8.1), in which we observed two distinct SSB-s.s. DNA binding modes possessing sites sizes of 33 and 65 nucleotides per bound SSB tetramer (Lohman & Overman 1985, JBC 260, 3594). Each of these binding modes differs in the number of nucleotides occluded upon interaction with s.s. DNA (i.e. site size). We have now identified a third distinct binding mode, at 25°C, possessing a site size of 56±3 nucleotides per bound SSB tetramer, which is stable over a wide range of $MgCl_2$ concentrations. At 37°C, a fourth binding mode is observed possessing a site size of 40±2 nucleotides per tetramer. A net binding of cations occurs upon formation of each of the higher site size SSB-DNA complexes. Mg^{++} is much more effective than Na^+ in facilitating the transitions to the higher site size binding modes. At 25°C (pH 8.1) the two binding mode transitions have midpoints of 0.6 mM and 64 mM in $MgCl_2$, and 17 mM and 0.16 M in NaCl, hence all three SSB binding modes may form in vivo since these salt concentrations are within the range estimated to occur in E. coli. These transitions also occur within the same range of $MgCl_2$ concentrations used for replication and recombination studies in vitro, hence they are of definite importance for any such studies. The relation between the protein fluorescence changes upon formation of a given binding mode and specific effects of cations and anions on the binding mode transitions will also be discussed. Since the E. coli SSB protein is essential for replication, recombination and repair processes it is possible that the different binding modes are used selectively in each of these processes in vivo.

M-Pos284 THERMODYNAMIC MODEL FOR THE BINDING OF E. COLI SSB PROTEIN TO S.S. DNA IN THE "BEADED", $(SSB)_{65}$ MODE. W. Bujalowski & T.M. Lohman, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843.
We have developed a statistical thermodynamic model ("octamer" model) which describes the equilibrium binding of the E. coli Single Strand Binding (SSB) protein to s.s. nucleic acids in its "beaded" binding mode [Chyrsogelos & Griffith (1982) PNAS U.S.A. 79, 5803], which seems to be equivalent to the high site size, $(SSB)_{65}$ binding mode [Lohman & Overman (1985) JBC 260, 3594]. The method of sequence generating functions is used to derive the model, which accounts for the observation that clustering of bound SSB tetramers is limited to the formation of octamers, which have been observed as "beads" in the electron microscope. The model also accounts for the overlap of potential protein binding sites on the nucleic acid and depends only upon three parameters; the site size, n, the intrinsic equilibrium constant, K, and the cooperativity parameter, ω. We obtain exact, closed form expressions for the binding isotherm as well as the distribution of bound SSB tetramers and octamers for this model, which allows one to easily calculate average binding properties and analyze experimental binding isotherms to obtain estimates of K and ω. In order to test the "octamer" model, we have determined the equilibrium binding isotherm for the E. coli SSB protein-poly(U) interaction in 0.2 M NaCl over a wide range of binding densities. These are conditions in which the low cooperativity, $(SSB)_{65}$ binding mode solely exists. The "octamer" model provides a much better description of the isotherm over the entire binding density range than models which assume that cooperativity results in the formation of clusters of unlimited size. A cooperativity parameter of ω=420±80, corresponding to an interaction free energy of -3.6 kcal per mole of SSB octamer formed, provides a good fit to data for SSB binding to poly(dA) and poly(U). Based on this moderate value of ω, the "octamer" model predicts that at low to intermediate binding densities, a significant fraction of bound SSB exists in the form of tetramers coexisting with octamers. In the case of E. coli SSB binding in the "beaded", $(SSB)_{65}$ mode this model provides a significant improvement over previous models which assume unlimited nearest neighbor interactions.

M-Pos285 ELECTRON MICROSCOPY OF THE SINGLE-STRAND DNA BINDING PROTEIN OF IKE VIRUS COMPLEXED WITH DNA. Carla W. Gray, Program in Molecular Biology, The University of Texas at Dallas, Box 830688, Richardson, TX 75083-0688.

The DNA binding protein encoded by filamentous IKe virus is known to have a 45% amino acid sequence homology with the widely studied gene 5 protein of the related fd virus, and detailed comparisons of the two DNA binding sites should provide a sensitive probe of structure-function relationships. As a prerequisite to the interpretation of such comparisons, high-resolution electron microscopy has been employed to determine whether the protein:DNA complexes formed by the two proteins are indeed similar.

IKe complexes were formed by titrating single-stranded circular fd viral DNA with IKe protein in 0.01 M NaCl, 0.01 M ammonium acetate pH 6.7. As with the fd protein, the IKe protein formed what appeared to be a loosely-coiled helical complex which presumably incorporates two single DNA strands (the "up" and "down" strands of the circular DNA) in a single-start nucleoprotein helix. The best estimate of the helix diameter (measured as a flat width) was 95 angstroms. The distance between helical turns varied, as in fd complexes, and thus the turns of the helix appeared to be somewhat flexible and not necessarily in close contact. For IKe complexes titrated to a nucleotide:protein ratio of 4:1, the mean helix pitch and total number of helical turns differed by 5% or less from values previously reported for fd complexes formed under slightly different conditions (C.W. Gray et al. (1982), Virology 116, 40-52). We conclude that the IKe and fd complexes are closely comparable in major aspects of their gross structures.

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M-Pos286 XENOPUS TRANSCRIPTION FACTOR IIIA BINDS TO THE NONSPECIFIC DNA SEQUENCES FLANKING THE INTRAGENIC CONTROL REGION OF THE 5S RNA GENE IN A HIGHLY ORDERED STATE.
William T. Windsor and C.-W. Wu, Dept. of Pharmacol. Sci., SUNY at Stony Brook, NY., 11794

Transcription factor IIIA (FA) from Xenopus oocytes is required for specific transcription of the 5S gene by RNA polymerase III. Purified FA has been shown previously by DNase I footprinting to bind specifically to an intragenic control region (ICR) that lies within the 5S gene. The structure of this specific FA-ICR complex is thought to be crucial for the exact location of transcription initiation. FA can also bind to nonspecific DNA though the affinity is some 50 fold weaker.

We have performed DNase I footprinting experiments with purified FA and the 5S RNA gene under conditions such that binding to specific and nonspecific DNA occurs. These conditions result in FA binding in a "highly ordered state" to nonspecific DNA flanking regions of the ICR. The presence of the ordered binding is indicated by an unique ~10bp periodic banding pattern present on either side of the ICR. This nonrandom banding pattern is similar to that expected for proteins bound to one side of double stranded DNA. Such binding may indicate the formation of a "histone like" nucleosome structure consisting of DNA wound around a protein core. This ordered binding by FA is not observed with DNA fragments consisting of only nonspecific DNA, suggesting that the specific protein-ICR complex is required as a nucleus to enable the ordered binding to occur. Concomitant with the ordered binding event is a change in the itragenic control region footprint of the coding strand such that the 3′ end of the ICR is fully protected but the 5′ end (~+45 to ~+72) has several new hypersensitive banding sites. The fact that new hypersensitive sites are formed within the ICR as well as the loss and formation of hypersensitive sites at ~+42 and ~+92bp, respectively, also suggests that protein-protein interactions exist between the ICR bound FA and adjacent nonspecifically bound FA molecules. The significance of these observations with respect to the mechanism of transcription will be presented. (Supported by NIH GM28069)

M-Pos287 STATISTICAL ANALYSIS FOR THE PREDICTION OF DNA - BINDING PROTEIN.
Kotoko Nakata, National Cancer Institute - F C R F, Frederick, Maryland 21701, U.S.A. and Minoru Kanehisa, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

DNA - binding proteins exhibit a number of characteristic patterns including homologous sequence and similar helix-turn-helix structures. We used this information in conjunction with a pattern recognition algorithm called the perceptron method. We made perceptron matrices for amino acid sequences of the proteins and for their original DNA sequences. Although the perceptron algorithm allows 100% discrimination between DNA - binding regions and other regions, its predictive ability, i.e. its ability to distinguish DNA - binding regions and other regions in sequences that are not in training set, is less reliable. By discriminant analysis we combine information processed by the perceptron algorithm with information obatained by other methods, e.g. distributions of hydrophobicity, hydrophilicity, charged amino acids, and three dimensional structures of protein and DNA, and improve the degree of predictive ability in unknown sequences. To asses the discriminatory power of these variables, we allocated additional sequences that have been newly included in the data base.

M-Pos288 THE fd GENE 5 PROTEIN BINDS TO DOUBLE-STRANDED POLY[d(A)]·POLY[d(T)]. Bi-Ching Sang and Donald M. Gray (Intr. by Dimitrij Lang). Program in Molecular Biology, The University of Texas at Dallas, Box 830688, Richardson, TX 75083-0688.

Circular dichroism data indicated that fd gene 5 protein formed a complex with double-stranded poly[d(A)]·poly[d(T)] (at 5mM Tris, pH 7.0, 20°C). The polymer showed an 8.8% decrease in absorption at 260nm and an altered CD spectrum at wavelengths above 255nm upon protein binding. These changes differed from those due to DNA denaturation. CD changes at both 268nm and 228nm revealed a titration endpoint at a [nucleotide]/[protein monomer] molar ratio of 4. The protein tyrosyl 228nm CD decreased 62% upon binding to double-stranded poly[d(A)]·poly[d(T)]. This reduction was significantly greater than that observed for binding to single-stranded poly[d(A)] (33%) or single-stranded poly[d(T)] (34%) but was similar to that observed for binding of the protein to double-stranded RNA (C.W. Gray, G.A. Page, and D.M. Gray (1984) J. Mol. Biol. 175, 553-559). The binding of gene 5 protein lowered the Tm of poly[d(A)]·poly[d(T)] by only 10°C (from 42°C to 32°C), even though the protein has been reported to lower the melting temperatures of other DNAs by about 40°C (B. Alberts, L. Frey, and H. Delius (1972) J. Mol. Biol. 68, 139-152). After the complex between the protein and poly[d(A)]·poly[d(T)] was melted, CD spectra showed that the protein formed complexes with the individual DNA strands. This process was slowly reversible, and the complex between the protein and polynucleotide duplex reformed when melted samples were kept at -20°C for one week.

This work was supported by NIH grant GM19070 and grant AT-503 from the Robert A. Welch Foundation.

M-Pos289 ^{13}C NMR OF REDUCTIVELY METHYLATED fd GENE 5 PROTEIN. Lawrence R. Dick, (*)A. Dean Sherry, and Donald M. Gray. (Intr. by Claud S. Rupert.) Program in Molecular Biology and (*)Program in Chemistry, The University of Texas at Dallas, Richardson, TX 75080.

Chemically modified derivatives of the fd gene 5 protein were prepared by reductive methylation of the protein amino groups with ^{13}C formaldehyde and sodium cyanoborohydride. (N. Jentoft and D.G. Dearborn (1983). Methods Enzymol. 91, 570-579.) This method permitted the incorporation of two ^{13}C-containing methyl groups on each of the six lysine ε-amino groups of the protein. The 50.1 MHz ^{13}C NMR spectrum of the protein was studied as a function of pH and salt concentration. Differences in the local chemical environments of the six lysyl residues allowed six corresponding resonances to be resolved in the pH range 8.5-9.0 at salt concentrations $\leq$ 50 mM NaCl. (Chemical shifts in ppm relative to external TMS were 43.33, 43.28, 43.17, 43.14, 43.05 and 42.78 at pH 8.76.) The resonance at 43.14 ppm was assigned to the C-terminal Lys87 by carboxypeptidase B digestion. Methylation of the protein while it was bound to poly[r(U)] yielded a selectively modified derivative in which one of the lysyl residues (43.05 ppm) was over 50% protected and one or two others at 43.33 and 43.28 ppm showed a lower level of protection. Thus, two or possibly three of the lysyl residues may be involved in the nucleic acid binding function. The resonance of one lysyl residue (42.78 ppm) showed pH-dependent behavior indicative of its involvement in an ion pair interaction (salt bridge). The N-terminal amino group of the protein reacted to a lesser extent than the lysine ε-amino groups at pH 7.5, and its resonance was unusually broad over the pH range 4-12, implying that it was not as fully solvated as suggested by the crystallographic structure. (G.D. Brayer and A. McPherson (1983). J. Mol. Biol. 169, 565-596.) (We appreciate support by NIH grant GM19060 and grants AT-503 and AT-584 from the Robert A. Welsh Foundation.)

M-Pos290 ^{1}H-NMR INVESTIGATION OF RELATIVELY HIGH MOLECULAR WEIGHT PROTEIN-DNA INTERACTIONS
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NMR methods are being used to further probe the roles of aromatic residues in the interaction of the fd gene 5 protein (G5P) and T7 RNA polymerase (T7RP) with their respective DNAs. In both cases, the interfacial nature of the interaction and the concomitant motional freedom of the surface groups involved permit the effective use of ^{1}H-NMR methods at quite high molecular weights.

For G5P (M_r 19.4 kd/dimer), evidence from several laboratories suggests that the details of a model for the G5P-ssDNA interaction originally proposed from a consideration of the G5P X-ray structure are incorrect. Contrary to this model, our NOESY studies on the binding of 4-12 mer oligonucleotides (complex M_r 20-60 kd) indicate that Tyr 41 does not interact significantly with nucleotide bases. In order to extend these studies to larger complexes (which some have proposed to be different to oligonucleotide complexes), the necessary spectral simplification has been achieved with the construction of an aro$^-$, his$^-$ host bacterium and selective deuteration, yielding only singlet Tyr (3,5)H resonances in the aromatic region of the G5P spectrum. In concert with site-directed mutagenesis, this approach is being used to examine the effects of G5P binding to 30-60 mer nucleotides.

Examination of T7RP (M_r 98 kd) binding to a synthetic DNA promoter sequence has begun recently. Use of the methods described above is expected to allow us to probe for aromatic amino acid-nucleotide base interactions, as well as to observe perturbations of the DNA base proton spectrum.

M-Pos291 STUDIES ON THE STRUCTURE OF THE ACTIVE SITE OF RNA POLYMERASE. P.P. Chuknyiski, J.M. Rifkind, E. Tarien and G.L. Eichhorn. Gerontology Research Center, National Institute on Aging, National Institutes of Health, DHHS, Baltimore, Maryland 21224.

Since the active site of E. coli RNA polymerase consists of an initiation site (IN) and an elongation site (EL), it is possible to map out the structure of the complete active site by studying the interrelationship between IN and EL. We make use of the fact that both IN and EL have metal ions bound to them - Zn(II) and Mg(II), respectively - and both of these ions can be replaced by Mn(II). The distances between Mn(II) on each site and substrate atoms on the same site have been previously determined in the laboratories of Mildvan and Wu and our own laboratory. Our previous studies of the EPR spectra of Mn(II) in these sites have shown that the spectra at each site at liquid helium temperature are considerably affected by Mn(II) in the other site. We observed diminution of the intensities of the EPR spectra. It was not possible to accurately determine the distance between these metals from this effect at 4°, since the evaluation of T_{1e} required for this calculation was not possible at that temperature. At room temperature, however, T_{1e} determination by NMR methods was feasible. The EPR spectrum of Mn(II) in EL, though less intense, was readily characterized, while the spectrum of Mn(II) in IN disappeared. From the effect of Mn(II) in IN on the EPR spectrum of Mn(II) in EL the distance between the two Mn(II) could be accurately calculated, and was found to be 5.2 A. This distance makes possible a precise formulation of the distances among the two metals and the two substrates in the active site. A model based on these distances leads to a mechanism for the translocation of substrate from EL to IN during RNA synthesis.

M-Pos292 UNIFIED NUCLEOSOME THEORY AND HISTONE ORGANIZATION. E.C. Uberbacher, J.M. Harp, and G.J. Bunick (intro. by B.E. Hingerty), University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, and Solid State Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

The X-ray crystal structure of the nucleosome core particle has been solved to 8 Å resolution in this laboratory, using molecular replacement supplemented with refinement based on density modification and four-fold non-crystallographic symmetry averaging. The histone portion of the nucleosome shows a low density interface between the H2A/H2B dimers and the centrally located tetramer. This interface not only makes the histone organization apparent, but provides the fundamental key to understanding the relationship between this structure and two other related structures — the core particle structure at 7 Å resolution, and the histone octamer structure at 3.3 Å resolution. The tripartite histone organization we see is similar to that seen in the histone octamer structure, although the two histone cores are not equivalent in size. Although the interface between the dimers and tetramers is apparent in our core particle structure, they are not separated by a significant channel, as they are in the octamer structure. In contrast, in the 7 Å resolution core particle structure, the interface between dimers and tetramer is not apparent, perhaps because of a somewhat tighter dimer to tetramer association. The relationship between these three structures will be described in detail. Possible dynamic mechanisms by which the nucleosome could "unfold" will also be discussed. (Research sponsored by Grant GM 29818 (to GJB) from the National Institutes of Health, and the Division of Materials Science, U.S. Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.)

M-Pos293 CU(II) AS A PROBE OF DNA ACCESSIBILITY IN CHROMATIN. Patricia Clark, Robin Roberson and Gunther L. Eichhorn (Intr. by Peter P. Chuknyiski). National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224.

When DNA is treated with Cu(II) and then heated, the melting temperature (T_m) of the DNA is dramatically decreased. The Cu(II) binds to the DNA in such a way as to destabilize the double helix and help to break the hydrogen bonds between the bases. When soluble chromatin is similarly treated with Cu(II) and heated, the T_m is unaffected. Apparently the Cu(II) cannot penetrate the chromatin structure and thus cannot initiate the DNA destabilization process. However, when H-1 histone is removed from the chromatin by affinity chromatography, subsequent treatment with Cu(II) does lead to a lowered T_m when the chromatin is heated. This T_m lowering is also achieved by two less drastic techniques that do not remove histidine H-1, but decrease the affinity of the H-1 to the DNA. These techniques are (1) a mild acetylation procedure that specifically modifies either 4 or 8 ε-amino groups of lysines on the H-1 histone, and (2) reaction with phosphate-binding divalent metal ions, e.g., Mg(II), Mn(II), or Co(II). In both of these instances the H-1 interaction with DNA is affected, and Cu(II) produces a decrease in the T_m of DNA. Apparently, removal of H-1 or decreased affinity of H-1 for DNA increases the accessibility of the DNA to the Cu(II). This phenomenon suggests a very simple probe for structural changes in chromatin produced by a change in the stability of the DNA - H-1 histone interaction.

M-Pos294 EFFECTS OF IN VIVO HYPERACETYLATION ON ISOLATED CHROMATIN CORE PARTICLES. L. J. Libertini, J. Ausio, K. E. van Holde, and E. W. Small, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA.

We have compared chromatin core particles containing hyperacetylated histones (ca. 17 acetyl groups per histone octamer) obtained from butyrate treated HeLa cell cultures to core particles obtained from untreated control cultures (ca. 4 acetyl groups per octamer).

Tyrosine fluorescence, sedimentation and circular dichroism all indicate that, at very low salt concentrations, acetylated particles undergo a transition at a considerably higher ionic strength than control particles. This result may indicate a weakening of the histone-histone or DNA-histone interactions which are disrupted at very low ionic strength. Alternatively, the increase in overall negative charge on the particle due to acetylation (from about -115 to -132) could be at least partially responsible.

Tyrosine fluorescence results indicate no significant differences in the high salt dissociation or in a subtle pH transition centered near pH 7. Furthermore, extensive digestion of the particles by micrococcal nuclease at 4 and 37°C also failed to reveal any differences in either the rate or pattern of DNA fragments produced. These results suggest that the points of acetylation are not involved in binding the DNA to the histone octamer. Considering the well known fact that stripped chromatin containing highly acetylated histones is digested by this nuclease to core particles at a much higher rate than control chromatin, we suggest that the points of acetylation may be concentrated in regions of the histone octamer which interact mainly with the linker DNA.

(Supported by PHS research grants GM 25663 and GM 22916.)

M-Pos295 PROTAMINE REPLACEMENT IN THE RAINBOW TROUT AS DETECTED BY ELECTRON SPECTROSCOPIC IMAGING. L. Aha, J.B. Rattner, D.P. Bazett-Jones. Intr. by F. Quandt.

During spermiogenesis in the rainbow trout, chromatin is completely reorganized as histones are replaced by a set of sperm-specific DNA-binding proteins, the protamines. The 10 nm polynucleosomal fiber undergoes a transition (involving several biochemical events) to a final 3 nm protamine-DNA fiber. The aggregation of protamine-DNA fibers and subsequent compaction of fiber "bundles" within the nucleus results in the highly condensed chromatin of the mature sperm.

Electron microscopic methods, including electron spectroscopic imaging (ESI), have been employed to characterize the type and distribution of chromatin fibers that occur at each stage of spermiogenesis. ESI enables the selective imaging of the distribution of phosphorus in electron microscopic specimens. The distribution of spread chromatin fibers in whole mounts of spermatogenic cells has been correlated with nuclear morphology in thin sections of developing testes. In spread chromatin, we have found an apparent morphological intermediate in the transition of the 10 nm polynucleosomal fiber to the protamine-DNA fiber. This 7 nm fiber, which appears smooth in conventional transmission electron micrographs, has a repeating unit of phosphorus density as detected by ESI. This structure is correlated with that of fragments of DNA containing hyperacetylated histones and may represent partially decondensed nucleosomes along the length of the fiber.

M-Pos296 **FREEZE FRACTURE STUDIES OF EXTREME THERMOACIDOPHILIC ARCHAEBACTERIA AND VESICLES FROM TETRAETHER AND RELATED LIPIDS.**

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Sulfolobus and Thermoplasma are two genera of archaebacteria which exist at high temperature and low pH. They are distinguished from mesophilic archaebacteria by having predominantly di-biphytanyl-tetraether lipids as the lipid component of their membranes. It is likely that these lipids confer on the membranes of thermoacidophiles the ability to withstand the extreme environments. In order to understand the structure and characteristics of these membranes, freeze fracture studies on the whole organisms, vesicles formed from tetraether lipids, and vesicles from a related synthetic lipid, 1,1 di-undecanoyl-2,2 [1,22docosanoyl]-diPC, have been performed. The fracture plane in membranes of Sulfolobus and Thermoplasma jumps completely across the membrane, whereas the fracture plane for Halobacteria, a mesophilic archaebacteria containing only diether lipids, is often found along the midplane of the membrane. Freeze fracture studies on vesicles from the isolated tetraether and related lipids should provide insight into the question of whether the structure in these vesicles is similar to that of the membranes in the extreme thermoacidophiles.

C.M. is a National Research Council-NRL Research Associate.

M-Pos297 CONFORMATION OF THREE PHOSPHATIDYLCHOLINE LIPIDS BY SOLID STATE CARBON -13 NMR: ESTER, ETHER AND AMIDE LINKAGES.

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Egg yolk lecithin, di-o-hexadecylphosphatidylcholine and sphingomyelin were aligned on glass cover slips and sealed within 10mm NMR tubes. Their orientation dependence with respect to the magnetic field was studied by solid state cross-polarisation carbon-13 NMR.

Using aligned lipids it was possible to resolve the chemical shift anisotropy of each type of carbon in the different lipids without the need for specific carbon-13 labelling. Dimyristoyl-phosphatidylcholine and di-o-tetradecylphosphatidylcholine were also studied to examine the effects of chain length on the chemical shift anisotropy.

Subtle differences in chemical shift anisotropy between the different lipids were interpreted in terms of conformation and dynamics, focusing particularly on the headgroup and backbone regions of the lipids.

M-Pos298 THE INTERACTION OF STRUCTURALLY RELATED LONG-CHAIN ALCOHOLS WITH PHOSPHOLIPID BILAYER MEMBRANES: A ^{2}H-NMR STUDY.

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The interaction of 1-dodecanol and 1-tetradecanol with dimyristoylphosphatidylcholine (DMPC) bilayers has been investigated as a function of temperature and alcohol concentration, by ^{2}H-NMR and DSC. Orientational ordering at many sites on DMPC, alcohol and water have been determined from quadrupolar splittings in the ^{2}H-NMR spectra. At equivalent reduced temperatures above the main phase transition the incorporation of either 1-dodecanol or 1-tetradecanol into the bilayer produces the same effects on orientational ordering in the phospholipid, at glycerol backbone, choline headgroup and acyl chain sites. Detailed C-^{2}H bond order profiles at several temperatures for 1-dodecanol and 1-tetradecanol were determined using selectively deuterated alcohols. A maximum order parameter is obtained for each alcohol at C4,5, with labels at both ends exhibiting reduced order. At equivalent reduced temperatures, the order profiles for 1-dodecanol and 1-tetradecanol were very similar. The effect of the two alcohols on water ordering in the vicinity of the main phase transition of DMPC bilayers at lower levels of hydration, is identical. In conclusion, our data support the view that the cut off in anesthetic potency observed in an homologous series of alcohols between n=12 and n=14, does not arise from any significant differences between 1-decanol and 1-tetradecanol, in their association with phospholipid bilayers.

M-Pos299 TARGET-SENSITIVE IMMUNOLIPOSOME AS AN EFFECTIVE ANTIVIRAL DRUG CARRIER IN INHIBITING HERPES SIMPLEX VIRUS REPLICATION. Rodney J.Y. Ho[1], Barry T. Rouse[2] and Leaf Huang[1]. Department of [1]Biochemistry and [2]Microbiology. University of Tennessee, Knoxville, Tn 37996-0840.

Employing free and liposome encapsulated antiviral drugs, the drug induced cytotoxicity and their ability to inhibit virus replication on the Herpes simplex virus (HSV) infected L929 cells was investigated. Among all the liposomes tested, the target-sensitive (TS) immunoliposomes composed of palmitoyl anti HSV and TPE (phosphatidylethanolamine transesterified from egg phosphatidylcholine) was most effective. When L929 cells were infected with 0.1 MOI, cytosine arabinoside (araC) encapsulated in the TS immunoliposomes could greatly reduce the cytotoxicity to the whole cell population while effectively inhibiting the virus replication. At 42 hr post infection, the concentration of araC at which 50% inhibition of ^{3}H-thymidine incorporation, CD_{50} (measurement of cytotoxicity) took place at 0.56 ng/ml for free drug and greater than 1 μg/ml for liposome encapsulated drug. While reducing the cytotoxicity, the araC encapsulated in the TS immunoliposomes effectively reduce the virus yield from the infected cells with ED_{50} of 100 ng/ml, whereas it required at least 100 fold higher concentration of free araC to achieve the same effect. Similar phenomena was also observed with the HSV specific anti-viral drug acyclovir (ACV). CD_{50} of the free and TS immunoliposome encapsulated ACV were detected at 12.5 ng/ml and 3 mg/ml, respectively; ED_{50} of the free and TS immunoliposome encapsulated ACV were 1.5 μg/ml and 100 ng/ml, respectively. The TS immunoliposome mediated delivery of the antiviral drugs may involve membrane bound nucleoside transporter, endocytosis, fusion or any combinations. (Supported by a grant from Liposome Sciences Inc., NIH grants CA24553 and AI14981).

M-Pos300 KINETIC AND ULTRASTRUCTURAL STUDIES OF INTERACTIONS OF TARGET SENSITIVE IMMUNOLIPOSOME WITH HERPES SIMPLEX VIRUS. R.J.Y. Ho[1], H Ping-Beall[2], B.T. Rouse[3] and L. Huang[1]. Department of [1]Biochemistry and [3]Microbiology, University of Tennessee, Knoxville, Tn 37996-0840. [2]Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

We have investigated the interaction kinetics between Herpes simplex virus (HSV) and target-sensitive (TS) immunoliposomes by following the light scattering and virus induced lysis of TS immunoliposomes. These liposomes, composed of phosphatidylethanolamine (PE) and stabilized by palmitoyl IgG against HSV glycoprotein D, were previously shown to release the aqueous content upon binding to the target HSV (Ho et al., Biochem. Biophys. Res. Commun. 1986, 38:931 and Biochemistry, 1986, in press). The HSV induced lysis of liposomes were detected continuously by monitoring the fluorescence enhancement of an encapsulated self quenching dye, calcein. Calcein leakage exhibited a biphasic kinetics. The rate of fast phase was strongly dependent on liposome and virus concentrations. This was followed by a second phase in which the rate of calcein release showed very little or no dependence on liposome concentration . Freeze-fracture (F-F) electron microscopy, employing plunge freezing technique, and negative stain electron microscopy showed extensive binding between HSV and TS immunoliposomes within the first min of incubation. From 30 sec to 3 min incubation time, we detected the HSV-liposome fusion intermediates in which the viral core protein appeared to be dissociated. After 6 min or longer, F-F electromicrograph showed extensive H_{II} phase that excluded the viral protein particles. Taken together, these studies indicated that HSV induced lysis of TS immunoliposomes involved the unstable fusion intermediate which eventually leads to the final non-bilayer H_{II} phase. (Supported by a grant from Liposome Sciences Inc., NIH grants CA24553 and AI14981.

M-Pos301 KINETICS OF MEMBRANE PHASE TRANSITIONS. W.W. van Osdol, Biophysics Program, University of Virginia, Charlottesville, Va. 22908

We are studying the kinetics of the main phase transition of model membranes by using a recently-developed volume perturbation calorimeter. The instrument functions by forcing a suspension of lipid vesicles to undergo small, adiabatic, bi-directional volume perturbations. The relaxation of the system to a new equilibrium state is monitored by measuring its temperature and pressure as functions of time after the application of the perturbation. Rate constants for the relaxation process are obtained by analyzing these observables in terms of sums of exponential decays. The instrument is capable of measuring apparent relaxations having characteristic times in the approximate range of 10ms to 10s.

Thus far we have examined single- and multi-lamellar dispersions of a single component, including phosphatidylcholines (PCs) or phosphatidylethanolamines (PEs) bearing identical, saturated acyl chains. We have also investigated the two-component systems PCs co-dispersed with the local anesthetic dibucaine. Our results for these systems will be presented.

The objective of our investigations is to better understand how the constituent parts of phospholipid molecules contribute to the dynamics of model and natural membranes.

M-Pos302 BILAYER DEFECTS AND SUSCEPTIBILITY TO PHOSPHOLIPASE A_2. S.W. HUI, T.V. ISAC AND A. SEN, ROSWELL PARK MEMORIAL INSTITUTE, BUFFALO, NEW YORK 14263.

The susceptibility to phospholipase A_2 was measured in mixtures of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and dilinoleyl phosphatidylethanolamine (DLPE). The aqueous suspension of this mixture is in the bilayer form at 35°C if the molar percentage of POPC is >10%. Below this percentage, the mixture is in the inverted hexagonal phase (H_{II}). Non-bilayer defects were detected by freeze fracture electron microscopy and by 31P NMR at the composition range of 10%-20% of POPC. Lightly sonicated samples of these mixtures were incubated with 3 units of phospholipase from porcine pancreas. The reaction reached saturation after 15 min. at 35°C. The products were analysed by thin layer chromatography and gas chromatography. Both the yield of hydrolysis and the ratio of hydrolysed PE/PC peaked at 15% of POPC, at which composition maximal non-bilayer defects were also found. The most susceptible state was not the bilayer-H_{II} transition but at the defect-laden bilayer form before the transition. PE seems to be preferentially attacked, presumably due to their location at or near the defect sites.

M-Pos303 DILATOMETRIC STUDIES OF THE KINETICS OF THE SUBGEL "C" PHASE TRANSITION IN DPPC. C.P. Yang, M. C. Wiener, S. Tristram-Nagle and J. F. Nagle. Departments of Physics and Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213.

The kinetics of formation of the subgel phase of DPPC have been reinvestigated by dilatometry. Volume decreases have been monitored at several incubation temperatures between 0.5 and 7 degrees C as a function of incubation time. Above 5°C the data clearly show a lag time that is consistent with nucleation limited transformation. The maximum temperature at which we have observed the transformation into the C phase has increased to nearly 7°C. Melting experiments performed on the subgel formed at different temperatures also indicate that there are two temperature regimes for forming the subgel. At very low temperatures, growth is rate limiting and at higher temperatures, nucleation is rate limiting. When growth is rate limiting, there are two peaks in differential scanning calorimetry traces when performed at ten degrees/hour, but when nucleation is rate limiting there is only one peak. A simple nucleation and growth model shows good agreement between the computer calculated results and the measured volume changes for the early part of the subgel formation. The latter part of the measured subgel formation cannot be continuously modelled with the initial part; the possible reasons for this are discussed.

M-Pos304 PHASE DIAGRAMS OF TWO-COMPONENT BILAYERS COMPOSED OF SYMMETRIC AND MIXED ACYL PHOSPHATIDYLCHOLINES. J. T. MASON. INTR. BY C. HUANG, UNIV. OF VIRGINIA, CHARLOTTESVILLE, VA 22908

High-sensitivity differential scanning calorimetry has been employed to study the thermotropic properties of two-component bilayers composed of the symmetric acyl phosphatidylcholine (PC) 1,2-distearoyl PC (C(18):C(18)PC) and sn-1 stearoyl derived mixed acyl PCs of progressively shorter sn-2 acyl chain length (C(18):C(16)PC, C(18):C(14)PC and C(18):C(12)PC). Phase diagrams were constructed from the onset and completion temperatures derived from the calorimetric transition profiles of these binary mixtures. The C(18):C(18)PC-C(18):C(16)PC system was found to form a nearly ideal mixture. The C(18):C(18)PC-C(18):C(14)PC mixture showed deviation from ideality but isothermal melting was not observed. However, the C(18):C(18)PC-C(18):C(12)PC system was strongly non-ideal displaying monotectic behavior. This is taken to indicate a phase separation of components in the gel phase of this bilayer system. This non-ideal behavior is believed to arise from the necessity to accommodate the dissimilar gel phase packing requirements of the C(18):C(12)PC and C(18):C(18)PC molecules in this two-component bilayer. (Supported by NIH grant GM-33040).

M-Pos305 DEPENDENCE OF THE FRACTION OF CHOLESTEROL UNDERGOING SPONTANEOUS EXCHANGE BETWEEN SMALL UNILAMELLAR VESICLES AND THEIR PHOSPHOLIPID COMPOSITION. L.K. Bar, Y. Barenholz and T.E. Thompson, Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908.

Spontaneous cholesterol exchange between small unilamellar vesicles comprised of different phospholipids and their binary mixtures has been studied in order to understand the factors involved in the establishment and maintenance of intracellular cholesterol distributions. Exchange was performed with neutral donor vesicles containing 10 mol% cholesterol, traces of [^{3}H]cholesterol and [^{14}C]cholesteryl oleate as a non-exchangeable marker. Acceptor vesicles in 10-fold excess had the same composition except 15 mol% phosphatidyl glycerol was included to input a negative charge to permit chromatographic separation of donor from acceptor vesicles. Data were best fitted by a single exponential and a base value. In donor vesicles containing only one phospholipid, the kinetic rate constants agreed with data reported previously, however the base values were larger than the expected equilibrium value of 9.09%. The size of this non-exchangeable pool was found to depend on the type of phospholipid. In binary phospholipid donor systems, well above the transition temperatures of the component lipids, the exchange parameters were closer to those of one of the pure components in the order of domainance: POPC > DMPC > DPPC > bovine brain SPM. Work supported by PHS grants GM-14628 and HL-17576.

M-Pos306 EFFECTS OF CHOLESTEROL ON THE STRUCTURAL TRANSITIONS INDUCED BY DIACYGLYCEROL IN PHOSPHATIDYL-CHOLINE (PC) AND -ETHANOLAMINE (PE) BILAYER SYSTEMS. Jens Coorssen and R.P. Rand, Biological Sciences, Brock University, St. Catharines, L233A1, Canada.

The membrane signal lipid diacylglycerol (DG) modifies and destabilizes phospholipid bilayers ultimately forming non-lamellar structures (Biochemistry 25:2882 (1986)). Since cholesterol (CH) forms a major fraction of many plasma membranes we have investigated, in closely paired samples, how it modifies DG's effect on PC and PE bilayers. We have characterized by X-ray diffraction the lamellar (L) or hexagonal (H) structures that form in excess solution. The table summarizes the mole ratios at which the L-L+H transitions begin, (which we interpret as those that destabilize bilayers), and at which the L+H-H transition is complete (which we interpret as the composition of the H structure). The mole % given reflects the amount of phospholipid that would have to be converted into DG to cause the indicated transition. For both phospholipids, cholesterol reduces the amount of DG required to destabilize the bilayer. For PE, the DG required to fully convert the lipid to H is unchanged but for PC is considerably increased. These and other effects suggest that in most cases the separate actions of DG and cholesterol on these bilayers are additive.

	L - L+H		L+H - H	
egg PE	95/0/5	5%	82/0/18	18%
egg PE + ch	65/33/2	3%	55/33/12	18%
egg PC	70/0/30	30%	65/0/35	35%
egg PC + ch	49/33/18	27%	35/33/32	48%

M-Pos307 Mechanism of Phase Transition Behavior in Phosphatidylcholine-Phosphatidylethanolamine Mixtures. B.A. Cunningham and L.J. Lis, Kent State Univ., Kent, Ohio; and P.J. Quinn, King's College London, U.K.

The components in lipid mixtures and biological membranes undergo phase transitions depending upon the localized domain structures within these systems. Preliminary experiments were done on a fully hydrated 4/1 dipalmitoylphosphatidylcholine (DPPC)/dipalmitoylphosphatidylethanolamine (DPPE) mixture to determine the structural consequences during phase transitions in real time. A high luminosity x-ray beam at the Daresbury (U.K.) Synchrotron Laboratory was used to obtain x-ray patterns, with temporal resolution of 150 ms to 1.5 sec, as a transition was being induced by a temperature scan or jump. Continuous x-ray patterns were recorded and analyzed during heating and cooling scans of approx. 10°C/min and a temperature jump of ~ 5°C/sec. We can infer that the L_a to L_β phase transition occurs via the formation of L_β micro domains in the L_a matrix, with the subsequent growth of the domains after the transition is complete. The transformation of the L_β bulk phase to the L_a bulk phase occurs directly upon heating. Phase transition behavior in other PC/PE mixtures will be discussed relative to these observations.

M-Pos308 Time-Resolved X-Ray Diffraction Study of the Mechanism of the Sub-transition in L-dipalmitoylphosphatidylcholine. B. Tenchov, Bulgaria Acad. Sci., Sofia, Bulgaria; L.J. Lis, Kent State Univ., Kent, Ohio, and P.J. Quinn, King's College, London, U.K.

Formation of the crystalline phase (L_c) of fully hydrated L-dipalmitoylphosphatidylcholine requires equilibration for several days at temperatures about 0°C. Transition from the L_c to the L_β phase is relatively rapid and can be observed as an endothermic process centered at a temperature of about 18°C. The structural changes associated with the phase change were examined using synchrotron X-rays and recording sequential diffraction patterns at 1.2 s intervals during heating at a rate of 5°C min^{-1}. Low-angle diffraction spacings showed changes in lamellar d-spacing indexed by four orders from 6.0 to 6.4 nm which closely followed the integral of the excess specific heat obtained by calorimetry performed under the same conditions. Characteristic changes in the wide-angle region of the diffraction pattern show a decrease in intensity of a sharp diffraction peak at 0.43 nm and a monotonic change in position of a broad diffraction band initially centered at 0.37 nm to an ultimate spacing of 0.41 nm characteristic of the L_β phase. The pattern of these changes in acyl chain packing from an orthorhombic hybrid subcell to an hexagonal subcell is characteristic of a second order phase transition. Temperature jump experiments at $6°.s^{-1}$ through the subtransition indicate that the transition time from L_c to L_β is approximately 5s. A subsequent transition from L_β to $L_{\beta'}$ is observed isothermally at 25°C after 12 min.

M-Pos309 Influence of Monovalent Ions on the Interactive Forces Between Phosphatidylcholine Bilayers. B.A. Cunningham and L.J. Lis, Kent State University, Kent, Ohio 44242.

Osmotic pressure and x-ray diffraction were used to measure the net repulsive pressure as a function of bilayer separation for dipalmitoylphosphatidylcholine in one molar solutions of monovalent salts (NH_4Cl, KSCN, KBr, KAcetate, K_2SO_4, and $BaCl_2$). The attractive and repulsive components of the net pressure were determined by first doing a least-squares fit of the observed pressure versus bilayer separation data to obtain the hydration pressure, and then equating the hydration and van der Waals pressures at the bilayer equilibrium separation in order to calculate a trial hamaker constant. Next, the van der Waals pressure was subtracted from the net pressure at each bilayer separation. The process was repeated until the hydration and van der Waals pressures converged. The average difference between experimental and calculated data points was approximately 1%. We calculated hydration pressure decay constants of 1.5 to 4.0 Å, and Hamaker constants for the van der Waals pressure of up to 50 x 10^{-14} ergs for DPPC bilayers in these salt solutions. The latter result indicates that an attractive hydration force due to the effect of certain monovalent ions (i.e., $Acetate^-$) on the polarization of the inter-bilayer water may be significant.

M-Pos310 MAGNETIC ORIENTATION OF SPHINGOMYELIN/LECITHIN BILAYERS. Julia B. Speyer[1]
Julia B. Speyer[1,2], P.K. Sripada[3], Sunil K. Das Gupta[2], G. Graham Shipley[3], and R.G. Griffin[2]. [1]Department of Chemistry and [2]Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139. [3]Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

Phospholipid bilayers consisting of a 60/40 mixture of N-palmitoylsphingomyelin and dimyristoyl phosphatidylcholine dispersed in 50 weight % water have been found to orient in strong magnetic fields. This orientation is easily observed in ^{31}P- and ^{2}H-NMR spectra where the intensity of the perpendicular edges of the powder lineshapes are enhanced at the expense of the parallel edges. Orientation occurs readily when the membrane is in the melted L_α-phase but will persist after cooling to the gel phase. The NMR lineshapes indicate that the lipids orient with their molecular long axis perpendicular to the field. The mechanism for orientation must involve either deformation of a spherical liposome into a prolate or oblate shaped liposome, or alignment of nonspherical liposomes in the magnetic field.

M-Pos311 ABSOLUTE SPECIFIC VOLUMES OF LIPIDS. M. C. Wiener, S. Tristram-Nagle and J. F. Nagle. Departments of Physics and Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213.

Measuring absolute specific volumes of lipids by the technique of D_2O/H_2O neutral buoyancy centrifugation is reexamined critically. In the past it has been assumed (1) that there is no preferential partitioning of deuterium into the interlamellar region, (2) that the volume of the water molecule in the interlamellar region is the same as in bulk water and (3) that deuterium does not affect the structure of the bilayers. The degree of validity of assumptions (1) and (3) is quantitated by control experiments using vibrating-tube densimetry and differential scanning calorimetry, respectively. Previous volumetric solution studies are used to discuss assumption (2). The resulting absolute specific volumes that are measured for DPPC, DLPE and for isobranched lipids are combined with recent x-ray diffraction results and hydration studies to yield a more complete picture of the structure of lipid bilayers. However, for the C phase of DPPC it is shown that there is an inconsistency in at least one reported measurement when subjected to our analysis. We also discuss two conflicting methods of obtaining the volumes of the methylene groups in fluid phases of alkanes and lipids.

M-Pos312 PHOSPHOLIPID HYDRATION FORCES: THEIR DEPENDENCE ON DIMENSION, SPECIES AND PHASE. R.P. Rand, Nola Fuller, Biological Sciences, Brock University, St. Catharines, 2253A1 Canada and V.A. Parsegian, DCRT, National Institutes of Health, Bethesda, MD 20892, U.S.A.

We have characterized in more detail the hydration force that phospholipid surfaces express in aqueous cavities. Water content is measured gravimetrically, water potential is controlled, osomtically, structural dimensions are characterized by X-ray diffraction. Head group contribution to hydration repulsion in lamellar phases is assessed by comparing POPE, SOPC, their mixtures, methylated trans-esterified eggPE, and diagalactosyldiglyceride. Highly variable hydration of different PE bilayers and disproportionate increase in hydration of modified PE bilayers suggests an extremely sensitive H-bonded water structure leading to hydration attraction within and between PE bilayers. Dependence on shape and size of the aqueous cavity is assessed by comparing the lamellar and hexagonal phases of POPE and DOPE, or of DOPE/DOPC 3/1 with and without tetradecane. Near full hydration the cylindrical aqueous cavities take up about twice the amount of water per polar group mass as the lamellar. Osmotic removal of that excess and the resultant changes in cavity curvature yields a measure of monolayer bending modulus. However near full dehydration hexagonal phases contain half the volume of water showing that the polar group-water "mash" is osmotically different in the two geometries.

M-Pos313 STATISTICAL MECHANICS OF ACYL CHAINS IN SATURATED AND POLYUNSATURATED BILAYERS FROM ^2H NMR. **Amir Salmon, Steven W. Dodd, and Michael F. Brown (Intr. by R. P. Taylor). Department of Chemistry and Biophysics Program, University of Virginia, Charlottesville, VA 22901.**

Deuterium (^2H) NMR studies have been conducted of disaturated phosphatidylcholines as well as mixed-chain, saturated-polyunsaturated phosphatidylcholines in the liquid-crystalline (L_α) phase. In both cases, the saturated acyl chain at the glycerol sn-1 position was labeled with ^2H by perdeuteration, whereas the sn-2 chain was unlabeled, i.e. protiated. The experimental ^2H NMR spectra were numerically deconvoluted (de-Paked) to obtain profiles of the segmental order parameters of the individual C-^2H bonds, denoted by $|S_{CD}^{(i)}|$, as a function of chain position. Significant differences in the ^2H NMR spectra and derived $|S_{CD}^{(i)}|$ profiles of the saturated-polyunsaturated and disaturated bilayers were found. Based on simplified statistical mechanical theories, the differences can be interpreted in terms of an increase in the configurational freedom of the saturated acyl chain at the glycerol sn-1 position in the mixed-chain polyunsaturated bilayers relative to bilayers of phosphatidylcholines with two identical saturated chains. The increased configurational freedom may correspond to a reduction of the lateral bilayer pressure or an increase in the mean area of the saturated acyl chains in the case of the mixed-chain polyunsaturated bilayers. We conclude that the configurational properties of the acyl chains of polyunsaturated bilayers are significantly different from those of saturated phospholipid bilayers. **Work supported by NIH Grants EY03754 and EY00255, the Jeffress Trust, and the Alfred P. Sloan Foundation.**

M-Pos314 A DEFORMABLE LATTICE MODEL FOR THE RIPPLE PHASE IN LIPID BILAYERS. H.L. Scott, Dept. of Physics, Oklahoma State University, Stillwater, OK 74078.

In the past theoretical studies of the ripple phase in lipid bilayers have been based upon two distinct approaches. One is thermodynamic in scope and involves construction and minimization of a phenomenological Free Energy. The other is a lattice model with molecules having competing orientational and displacement perpendicular to the plane interactions. While the lattice model does exhibit a chain-tilted ground state, a modulated (ripple) phase, and a disordered phase, the Hamiltonian requires a large number of configurational energy parameters. Although one needs only the relative sizes of these parameters to determine the phase diagram, it is not possible to evaluate them even at the relative level very precisely. In this paper a new approach will be presented which combines some aspects of both of the earlier methods. The model to be presented is a two dimensional lattice model with orientational degrees of freedom for the molecules and with continuous harmonic degrees of freedom for motion perpendicular to the lattice plane. The model Hamiltonian, which is much simpler than the earlier lattice model, may in some cases be rewritten via a variable change so that orientation and harmonic degrees of freedom are decoupled. Then the problem reduces to that of a 2-D Ising Model with many-spin interactions. The results of preliminary mean field and Monte Carlo studies of the model will be presented and compared to experimental phase diagrams.

M-Pos315 WATER BINDING SITES IN FULLY HYDRATED DPPC BILAYERS INVESTIGATED BY HIGH-PRESSURE INFRARED SPECTROSCOPY.

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We have recently demonstrated that the formation of hydrogen-bonds between membrane lipids and molecules containing proton donating groups, such as water or cholesterol, can be detected by use of high-pressure infrared spectroscopic techniques (1,2). In the present study we have applied this technique to investigate the possible binding sites of water molecules to dipalmitoyl phosphatidylcholine (DPPC) in fully hydrated DPPC bilayers. Evidence is given to show that among all the polar groups of the head group and the interfacial region, water molecules are strongly hydrogen-bonded to the PO_2^- group in the head moiety and weakly hydrogen-bonded to the ester C=O group in the interfacial region of DPPC. No hydrogen-bonds were detected between water and the choline head group or the C-O-C ester group of DPPC. A polar interaction between water and the choline moiety in the head group region is observed.

1. E.C. Mushayakarara, P.T.T. Wong and H.H. Mantsch, Biochem. Biophys. Research Comm. 134, 140-145 (1986).
2. E.C. Mushayakarara, P.T.T. Wong and H.H. Mantsch, Biochim. Biophys. Acta., 857, 259-264 (1986).

M-Pos316 **EFFECT OF HYDROSTATIC PRESSURE ON THE TRANSMEMBRANE DIFFUSION OF PHOSPHATIDYLETHANOLAMINE.** Reynold Homan and Henry J. Pownall, Baylor College of Medicine, Houston, Texas 77030

The mechanism of spontaneous transmembrane diffusion (flip-flop) of phospholipids is not well understood. Phospholipid flip-flop involves inversion of the diffusing lipid with passage of the headgroup through the membrane interior. We have measured the flip-flop rates of pyrene-labeled phosphatidylethanolamine (PE) as a function of hydrostatic pressure to determine the size and sign of the volume change associated with migration of PE across the bilayer of sonicated phosphatidylcholine vesicles. At 50°C, PE flip-flop rates decreased with pressures increasing to 200 MPa. The calculated volume of activation was 19 cm^3mol^{-1}. Addition of cholesterol (10 mol%) to the vesicles reduced the flip-flop rate at atmospheric pressure (~ 0.1 MPa) from $4 \cdot 10^{-3}s^{-1}$ to $3 \cdot 10^{-3}s^{-1}$. Furthermore, the activation volume for flip-flop in vesicles containing cholesterol was 5 cm^3mol^{-1}. The positive volumes of activation are consistent with a model in which neighboring phospholipids must be displaced to accommodate the inversion and translocation of a phospholipid species across the bilayer. Factors which reduce the free volume within the bilayer, such as hydrostatic pressure and cholesterol, inhibit phospholipid flip-flop. The lower activation volume for PE flip-flop in vesicles containing cholesterol may be due to reduction of the lateral compressibility of the bilayer by cholesterol.

M-Pos317 LIPID TRANSFER REACTIONS UNDER PRESSURE PERTURBATION. W. W. Mantulin. Laboratory for Fluorescence Dynamics, Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

In cells, the transport of lipophilic molecules amongst various compartments represents an important processing mechanism. Spontaneous lipophile transfer between membrane surfaces is governed by the molecular structure of the transported lipophile and its microenvironment. The time course of excimer intensity decay from transported pyrene-labeled lipid analogs provides a direct measure of the spontaneous desorption event. In general, such measurements have indicated a spontaneous transfer mechanism whose rate limiting step involves lipophile desorption and solubilization in the aqueous phase; subsequently, uptake by an acceptor membrane occurs on a slower timescale. Perturbation by high hydrostatic pressure does not alter this mechanism, but does retard the transfer process. The activation volume ($\Delta V\ddagger$) for the transport event is positive. For a homologous series of pyrene-labeled phospholipids ($\Delta V\ddagger$) increases with molecular size. For example, the $\Delta V\ddagger$ for 1-myristoyl-2 [9'(3'-pyrenyl)nonanoyl] phosphatidylcholine (MPNPC) transfer is approximately 12 ml/mol, whereas it is 9 ml/mol for the corresponding lauryl (LPNPC) derivative. Aqueous viscosity does not alter the transport process, but it is dependent on compressibility of the host lipid matrix. Hydrostatic pressure rigidifies membranes and this loss of fluidity can be detected by fluorescence methods. However, there is no correlation between membrane fluidity and lipophile transport. Supported by 1P41-RR03155.

M-Pos318 HIGH PRESSURE VIBRATIONAL SPECTROSCOPIC STUDIES OF THE STRUCTURE AND PACKING OF UNSATURATED CHAINS IN LIPID BILAYERS. D.J. Siminovitch, P.T.T. Wong and H.H. Mantsch. Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6.

Infrared and Raman spectra of aqueous dispersions of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dielaidoyl-sn-glycero-3-phosphocholine (DEPC) were measured in a diamond anvil cell at 28°C as a function of pressure up to 40 kbar. The infrared spectra indicate that DEPC and DOPC undergo pressure-induced liquid crystalline to gel phase transitions at critical pressures of 0.7 kbar and 5.2 kbar, respectively. Below their respective critical pressures, the infrared spectra of DOPC and DEPC are essentially indistinguishable, whereas above these pressures, there are very pronounced differences in the barotropic behaviour of these two lipids. Specifically, at the 5.2 kbar transition in DOPC, there are significant changes in the frequencies, intensities and widths of vibrational bands associated with the C=O groups, the CH=CH groups and the terminal methyl groups, whereas the corresponding bands of DEPC are, by contrast, strikingly insensitive to the pressure-induced phase transition. The unusual band shape changes in DOPC are attributed to a unique packing arrangement of the oleoyl chains required to accommodate the bent geometries of adjacent cis-double bonds. Moreover, above 5 kbar in DEPC, well-defined correlation field splittings of the CH_2 scissoring and rocking modes are observed, with magnitudes very similar to those observed at comparable pressures in saturated lipid systems. The absence of correlation field splittings of the corresponding bands of DOPC up to 40 kbar suggests that the bent oleoyl acyl chains are closely packed with all chains oriented parallel to each other.

M-Pos319 INFLUENCE OF SURFACE PRESSURE ON PARTITIONING OF HYDROPHOBIC MOLECULES INTO MEMBRANES. Robert C. MacDonald, Departments of Biochemistry, Molecular Biology and Cell Biology and of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

It is generally assumed that when hydrophobic molecules partition into a lipid bilayer, the entire volume of the hydrophobic core is available to them. This assumption is shown to be invalid; given ideal mixing, unless a molecule has very high surface activity, it is strongly excluded from regions other than the center of the bilayer. Consider a fluid phase phosphatidylcholine bilayer: At room temperature, each of the constituent monolayers exerts a surface pressure, π, of about 50 dynes/cm. If a permeant molecule enters such a monolayer and resides anywhere near the polar interface, the monolayer will expand. Surface pressure resisting entry of the penetrating molecule is $\Delta\pi = 50 - \pi_e$, where π_e signifies the equilibrium surface pressure of that molecule at the concentration of interest. Residence between alkyl chains will hence incur a free energy cost equal to the product of $\Delta\pi$ and the partial molar area of the penetrating molecule, i.e., $\Delta\pi a$. The driving force for penetration and residence at such a site is the free energy of mixing, $RT\ln X$, where X is the mole fraction of the penetrating molecule in the bilayer, so, at equilibrium, $\Delta\pi a = -RT\ln X$. Given a = 25 $Å^2$/molecule, the calculated partition coefficients vary by a factor of about 300 over the range of equilibrium pressures possible for the partitioning molecule, namely, 0-50 dynes/cm. Thus, molecules with the same oil/water partition coefficient may vary greatly in their intramembrane location, some residing within monolayers and others between monolayers. Unlike a thin sheet of oil, a bilayer constitutes two interfaces, all positions of which are occupied, and newcomers must be surface active in order to squeeze in. Supported by NIH grant NS20831.

M-Pos320 MEMBRANE SURFACE TENSION MEASUREMENT WITH AMPHIPATHIC DYES, Rodman G. Miller and Linda Y. Chow, Department of Anatomy, University of Calgary, Alberta.

Fluorescence intensity of dyes are dependent upon the dielectric constant of their immediate environment. Amphipaths alter their partitian coefficient dependent upon the surface tension at an interface. The surface tension of closed biological membranes can be altered by establishing an osmotic gradient across the membrane. We have used this reasoning to develop a fluorometric assay for membrane surface tension.

Several dyes were tested for dependence of fluorescent intensity with changes in osmotic gradient. Fluorescence intensity of N-(3-sulfopropyl)-4-(p-dibutylamino-styryl) pyridinium (di-4-ASPPS, Molecular Probes, OR) is a function of osmotic gradient across both liposomal membranes and sealed red blood cell ghosts. Di-4-ASPPS was added to liposomes prepared by rapid extrusion, loaded with 450 mM sucrose in 20 mM phosphate buffer and a gradient was established by dilution of the liposomes with varying concentrations of sucrose in buffer. Fluorescence intensity was found to increase linearly by approximately 70% under conditions of external sucrose concentrations less than 350 mM. Creation of flacid liposomes (external sucrose concentration more than 350) resulted in little change in the fluorescence intensity. Red blood cell ghosts loaded with 350 mM sucrose in buffer demonstrated a similar increase in fluorescence intensity with decreasing external osmotic media.

These results suggest that di-4-ASPPS is capable of reporting membrane surface tension. In the future, this method will be utilized to measure the surface tension of the external leaflets of plasma membranes and membrane organelles. This work supported by NSERC and Canadian Cystic Fibrosis Foundation.

M-Pos321 CHARACTERIZATION OF THE ETHANOL INDUCED TRANSITION OF DPPC FROM THE BILAYER GEL TO THE INTERDIGITATED GEL PHASE. Jeffrey A. Veiro and Elizabeth S. Rowe. University of Kansas Medical School, Kansas City, KS, and the VA Medical Center, Kansas City, MO.

A wide range of surface active molecules including ethanol have been shown by X-ray diffraction to induce a fully interdigitated gel phase in dipalmitoylphosphatidylcholine multilamellar liposomes [McIntosh, T.J., McDaniel, R.V. and Simon, S.A. (1983) Biochim. Biophys. Acta. 731, 109-114]. We have recently shown using spectrophotometric techniques that the simultaneous appearance of three phase transition related phenomena is correlated with the presence of this interdigitated phase. These phenomena are: 1) an abrupt fractional decrease and ultimate disappearance of the pretransition; 2) onset of thermal hysteresis in the main phase transition; 3) an inflection in the ligand dependence of the main transition temperature.

In this report the very narrow ligand concentration range where the fractional decrease in the magnitude of the pretransition occurs is extensively investigated. It is found that ethanol and other short chain n-alcohols initiate the appearance of a double transition in this concentration range. One of the transitions exhibits significant thermal hysteresis while the other is reversible. It is suggested that the additional transition is representative of a proportion of the liposomal suspension present in the interdigitated phase. The results are discussed in terms of alcohol induced lateral phase separation of the bilayer gel and interdigitated gel phase. (Supported by the Veterans Administration and NIAAA)

M-Pos322 RESTRICTION OF LATERAL DIFFUSION IN LIPID MEMBRANES BY SACCHARIDES. George Strauss, Dept. of Chemistry, Rutgers University, New Brunswick N.J. 08903.

Small unilamellar phospholipid vesicles fuse into large multilamellar aggregates when dehydrated or frozen. This structural rearrangement is suppressed by mono-or disaccharides (Strauss and Hauser (1986), PNAS 83, 2422). To clarify the mechanism involved, lateral diffusion coefficients in the membranes were determined by measuring fluorescence lifetimes as a function of the sucrose concentration in the bulk aqueous phase. The vesicles carried pyrene or pyrene derivatives as fluorophores, and nitroxide free radicals as quenchers, with their reactive groups at fixed depths from the membrane interfaces but free to move laterally. When the groups were in the polar interfacial region (pyrene-DPPE or pyrenesulfonylhexadecylamine plus TEMPO-hexadecanoate) the quenching rate constant began to drop beyond 20% sucrose and at 60% sucrose had decreased to 67% of the sugar-free control. Sucrose had a similar effect when the fluorophore and quencher moieties were in the membrane interior but attached to long-chain probes having polar groups at the interface (16-pyrene-hexadecanoic acid plus 16-doxylstearic acid methyl ester). Sucrose had no effect on the quenching rate when the fluorophore was free pyrene, located in the membrane interior and independent of the interface.

It is concluded that sucrose and other sugars stabilize lipid membranes by binding to the polar headgroups, thus stiffening the interface, and that such binding is cooperative.

M-Pos323 TREHALOSE INHIBITS LAMELLAR TO HEXAGONAL PHASE TRANSITIONS IN PHOSPHATIDYLETHANOLAMINES. Christina Aurell Wistrom, Lois M. Crowe, Barry J. Spargo, and John H. Crowe, Department of Zoology, University of California, Davis, CA 95616

Formation of a non-bilayer, inverted hexagonal phase by phosphatidylethanolamines has long been supposed to be a major source of damage to biological membranes during dehydration or freezing. However, such non-bilayer phases appear to be lacking in organisms that normally survive dehydration. We present here a mechanism by which formation of hexagonal phase lipid may be prevented in these organisms. Many such organisms contain high concentrations of trehalose, which has previously been shown to stabilize intact biological membranes and vesicles of pure phospholipids in the absence of water. In the present study, we show that trehalose can inhibit the thermotropic phase transition of hydrated pure natural and synthetic phosphatidylethanolamines from lamellar liquid crystalline to hexagonal phase. Differential scanning calorimetry and Fourier transform infrared spectroscopy show that in the presence of trehalose, dioleoylphosphatidylethanolamine and egg phosphatidylethanolamine do not undergo a transition to hexagonal phase for at least 20-70° C above their normal phase transition temperature. A model of the effect of trehalose on phosphatidylethanolamines is presented. (Supported by grant DMB 85-18194 from NSF).

M-Pos324 MODES OF INTERACTION OF CRYOPROTECTANT WITH MEMBRANE PHOSPHOLIPIDS DURING FREEZING. Thomas J. Anchordoguy, Alan S. Rudolph, John Carpenter, and John H. Crowe, Department of Zoology, University of California, Davis, CA 95616

The abilities of a variety of compounds to inhibit liposome fusion during freeze/thaw were assessed by resonance energy transfer. Small unilamellar vesicles have been frozen according to three different protocols. Membrane intermixing was seen to be relatively independent of freezing protocol except when glycerol, DMSO, or sarcosine were used as the cryoprotectant. Low concentrations of polyvinylpyrolidone or 4-hydroxyproline enhanced fusion of liposomes, whereas high concentrations of these compounds had no effect. Glycerol, DMSO, proline, betaine, and sarcosine reduced fusion, but only when their concentrations were greater than 1M. The most effective cryoprotectants were trehalose and sucrose, which both reduced fusion to minimal levels at concentrations of only 0.2M. We have also used europium to probe the modes of interaction of these compounds with phospholipids. Europium, which is known to bind to the phosphate headgroup, maximized fusion in liposomes subjected to freeze/thaw. This "europium-induced" fusion was progressively reduced by the presence of increasing sucrose, trehalose, or glycerol, suggesting a competition for the headgroup. However, the presence of proline, betaine, or sarcosine did not reduce europium-induced fusion, suggesting that these compounds do not compete for the headgroup. Substitution of polar side chains on the hydrophobic regions of proline or betaine eliminate their cryoprotective properties, suggesting that these compounds interact with the acyl chains of the bilayer. (Supported by grant DMB 85-18194 from NSF).

M-Pos325 PRESERVATION OF DRY LIPOSOMES DOES NOT REQUIRE RETENTION OF RESIDUAL WATER. John H. Crowe, Barry J. Spargo, and Lois M. Crowe, Department of Zoology, University of California, Davis 95616

Certain sugars, particularly trehalose, dramatically alter physical properties of dry phospholipids in ways that mimic the presence of water. As a result, these sugars are capable of preserving the integrity of dry liposomes and membranes. Since these effects could conceivably be due to the presence of small amounts of water in the dry preparations of sugar and lipid, we have done careful measurements of the residual water contents in the dry samples and report the results here. Lyophilized liposomes composed of palmitoyloleoylphosphatidylcholine and phosphatidylserine (9:1) contain at most 0.2 mole water/mole lipid. When the trehalose concentration in the dry mixtures is increased, there is no increase in the apparent water content of the samples over a wide range of sugar concentrations. Over the same range of trehalose contents the maximal effect of trehalose on physical properties (including thermotropic phase transitions measured with differential scanning calorimetry and Fourier transform infrared spectroscopy) of the lipids is achieved. Similarly, maximal stabilization of liposomes with respect to retention of trapped solute during drying and rehydration is realized over this range of trehalose contents. We conclude that the stabilization does not require retention of residual amounts of water in the dry trehalose-phospholipid preparations. Similar studies with other sugars show a relationship between the amount of sugar interacting with the lipid and the ability of the same sugar to stabilize dry liposomes. (Supported by grant DMB 85-18194 from NSF).

M-Pos326 STRUCTURAL CHARACTERIZATION OF MELITTIN-PHOSPHATIDYLCHOLINE DISC LIKE COMPLEXES. M. Lafleur and M. Pézolet, Département de chimie, Université Laval, Québec, Qc, G1K-7P4, Canada.

From the results obtained from electron microscopy, quasi-elastic light scattering and NMR spectroscopy, it was concluded that melittin destroys the multilamellar structure of phosphatidylcholine dispersions and lead to the formation of small particles. Our Raman results provide valuable information on the structure of these particles. In the gel state, melittin induces a drastic decrease of the h_{2880}/h_{2850} intensity ratio which indicates that the interchain vibrational coupling is significantly lowered. However, the intramolecular order of the lipid acyl chains, measured from the C-C stretching mode region, is much less affected by the presence of the toxin. The thermotropic behavior of these complexes shows a shift of the gel to liquid-crystalline phase transition toward the low temperatures. The cooperativity of this transition increases with the chain lenght of the phosphatidylcholine. Therefore, we propose that, as for apolipoproteins, melittin induces the formation of small PC bilayer discs whose hydrophobic edges are shielded from the aqueous phase by a layer of toxin. In such a "bicycle tire" like structure, the chains can remain in almost all-trans conformation but due to the small size of the discs, there is less intermolecular coupling and a decrease of the cooperativity of the phase transition. The perturbation by the toxin on phosphatidylcholines with longer chains is smaller due to the higher cohesion of the hydrophobic core.

M-Pos327 **CALORIMETRIC CHARACTERIZATION OF THE THERMAL AND pH STABILITY OF DIPHTHERIA TOXIN.** Glen Ramsay and Ernesto Freire, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218

The thermodynamic stability of diphtheria toxin has been investigated by high sensitivity differential scanning calorimetry as a function of pH, in the absence and in the presence of phospholipid vesicles composed of egg PC:DPPA:Cholesterol (5:1:3). At neutral pH and in the absence of lipid vesicles diphtheria toxin undergoes thermal denaturation at 56 °C with a total enthalpy change (ΔH) of 330 kcal/mol. This transition is not a two-state process and can be dissected into two separate components with enthalpies of 130 and 200 kcal/mol respectively, suggesting that the A (MW=21,145) and B (MW=37,240) domains of the toxin behave independently. The low enthalpy transition appears to be a two-state process (ΔHvh/ΔHcal=1.05) whereas the high enthalpy transition is characterized by a ΔHvh/ΔHcal of 0.5. At slightly acidic pH (pH=6) the high enthalpy transition is shifted downwards (Tm=46 °C) and becomes very broad (ΔHvh/ΔHcal=0.21). Finally at pH=4 only one peak centered at 30 °C and with a ΔH of 140 kcal/mol remains. These experiments suggest that the pH induced transition observed at 20 °C corresponds to the high enthalpy component in the thermal denaturation profile. In the presence of phospholipid vesicles, the calorimetric scans are characterized by the presence of an additional broad exothermic peak at ~60 °C. The exothermic peak has a ΔH of-500 kcal/mol of toxin and its magnitude is independent of pH under the conditions of these experiments. At neutral pH this exothermic peak follows the onset of thermal unfolding of the toxin and could be related to the membrane insertion of the protein, even though at this time no positive assignment has been made. (Supported by NIH grant GM-37911).

M-Pos328 FACTORS AFFECTING THE MEMBRANE TRANSLOCATION OF THE A_1 SUBUNIT OF CHOLERA TOXIN. Can Bruce and Bernadine J. Wisnieski. Department of Microbiology and the Molecular Biology Institute, University of California, Los Angeles, CA 90024.

In this study we have examined how temperature, pH and reducing agents affect the membrane penetration behavior of the A_1 subunit of cholera toxin (CTx). CTx is secreted as A5B and subsequently cleaved to $A_1A_2$5B. Reduction of the disulfide bridge between A_1 and A_2 leads to induction of the ADP-ribosylation activity of the A_1 subunit. Toxin targets consisted of large unilamellar vesicles (e.g., dimyristoylphospatidylcholine plus or minus the ganglioside receptor GM_1) prepared by the reverse phase evaporation method. The photoreactive probe 12-(4-azido-2-nitrophenoxy)stearoyl[1-^{14}C]glucosamine was used to monitor membrane penetration. An in vitro ADP-ribosylation assay was developed for use with vesicle targets to examine acquisition of ADPr-transferase activity of membrane-bound toxin. Vesicles loaded with trypsin were used in conjunction with unloaded vesicles and exogenous trypsin treatment to explore the location of the trypsin cleavage site of the intact A subunit after toxin binding. Results show that reduction is not necessary for insertion of the A_1 domain. Kinetic studies indicate that with fluid targets, toxin insertion reaches a maximum in <1 minute. The peptide bond that connects the A_1 and A_2 domains of A appears to remain outside the bilayer after A insertion is achieved. This work was supported by USPHS grant GM22240, the UCLA Academic Senate and an Ursula Mandel Scholarship (CB).

M-Pos329 THERMOTROPIC BEHAVIOR OF CHOLERA TOXIN AND CHOLERA TOXIN-GANGLIOSIDE Gm1 MIXTURES. Beth Goins and Ernesto Freire, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

High sensitivity differential scanning calorimetry and differential solubility thermal gel analysis have been used to characterize the interaction of cholera toxin with its receptor ganglioside Gm1. In the absence of ganglioside Gm1, cholera toxin undergoes two well defined thermally induced transitions. The first transition, centered at 51 deg C (ΔH = 76 kcal/mole) has been assigned to the irreversible unfolding of the A subunit. The second transition, centered at 74 deg C (ΔH = 520 kcal/mol of toxin) represents the denaturation of the B subunit pentamer. Immediately following the onset of unfolding of the B subunit pentamer (70 deg C) an exothermic heat effect is present in the calorimetric scans. This exothermic effect is not present upon rescanning of the samples and has been shown by gel analysis to arise from the dissociation and precipitation of the A subunit from the B pentamer. Binding of the toxin to micellar ganglioside Gm1 or ganglioside Gm1 incorporated into lipid vesicles abolishes the exothermic effect, causes a 20 deg C upward shift in the melting temperature of the B pentamer but has no effect on the transition temperature of the A subunit, indicating that neither ganglioside Gm1 nor the toxin B subunit influence the stability of the A subunit. These experiments support a model by which the insertion of the A subunit into the membrane is preceded by a partial unfolding of the B subunit pentamer that exposes the A subunit to the membrane surface. (Supported by NIH grant NS-24520)

M-Pos330 EFFECT OF LIPID COMPOSITION UPON DIPHTHERIA TOXIN BINDING TO MODEL MEMBRANES. Laura A. Chung and Erwin London, Dept. of Biochemistry S.U.N.Y at Stony Brook, Stony Brook, NY 11794-5215.

The effect of pH upon the binding of diphtheria toxin to small unilamellar model membrane vesicles was determined using fluorescence quenching. It was observed that as pH was decreased the toxin underwent a sharp transition from a state that binds to lipids negligibly, to one in which binding was tight and accompanied by deep penetration of the protein into the fatty acyl chains. A series of studies were undertaken to determine the effect of lipid composition upon the binding of lipids to the toxin. It was shown that toxin binds to zwitterionic vesicles only slightly less than to anionic vesicles. However, the transition pH, which is the point at which toxin binding becomes significant, depended upon the fraction of anionic lipids, being highest in model membranes composed totally of anionic lipids (pH 5.8), and lowest in membranes composed of zwitterionic lipids (pH 5.1). Aside from charge, transition pH was independent of the nature of the lipid polar headgroups studied. It was found that high ionic strength shifts the transition with anionic vesicles to about pH 5, but did not affect the transition pH with zwitterionic vesicles. These experiments suggest that the ionic double layer, which sets up a low local pH around anionic vesicles, alters the apparent pH, but not the intrinsic pH, at which the toxin becomes hydrophobic and inserts. Tighter and more rapid binding of toxin to vesicles containing anionic lipids was also observed. This is probably due to electrostatic interactions. These results suggest that binding at low pH is more a result of hydrophobic interactions than of specific toxin-lipid headgroup interactions in many cases, but a stronger interaction of toxin with anionic lipids as compared to zwitterionic ones cannot be ruled out. Supported by NIH Grant GM31986.

M-Pos331 LIPID STRUCTURE AT PROTEIN SURFACES AND LIPID STRUCTURAL CHANGES CORRELATING WITH THE BILAYER TO HEXAGONAL II PHASE TRANSITION AS REVEALED BY RAMAN SCATTERING. A. K. Dunker and K. W. Short, Department of Chemistry and Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164-4660.

Distearoyl phosphatidyl choline (DSPC) converts from the bilayer to the hexagonal II phase when added gramicidin exceeds a critical value; dimyristoyl phosphatidyl choline (DMPC) does not convert at any gramicidin proportion. We have used laser Raman scattering to study the lipid acyl chain structure in DSPC and DMPC as a function of gramicidin concentration. In contrast to NMR and ESR, Raman scattering provides a direct measure of lipid acyl chain conformation.

Both DMPC and DSPC show a marked increase in gauche bonds as the concentration of gramicidin is increased. We suggest that the decrease in chain order is due to the bending of the lipid tail groups as they conform to the rough surface of the gramicidin molecule. These results are in stark contrast to our earlier results showing that B. A. Wallace's DMPC/gramicidin co-crystals contain two highly ordered lipid molecules/gramicidin. One possibility is that the gramicidin surface has two locii for ordered lipids and several places for highly disordered lipid tails.

DSPC, but not DMPC, shows an additional decrease in lipid acyl chain order at high proportions of gramicidin, corresponding to the formation of hexagonal II phase. We suggest that the formation of hexagonal II phase leads to a substantial increase in the number of gauche bonds by allowing rotation of C-C bonds that are close to the lipid head group.

M-Pos332 MIXED GRAMICIDIN-LIPID MONOLAYERS. N. Davion-van Mau[1], P. Daumas[1], Y. Trudelle[2], and F. Heitz[1] (Intr. by O. S. Andersen). 1: Laboratoire de Physicochimie des Systemes Polyphases, BP 5051, F-34033, Montpellier Cedex, France; 2: Centre de Biophysique Moleculaire, CNRS, F-45071, Orleans Cedex 2, France.

Gramicidin A (GA) and its destryptophylphenylalanyl analogue (GM) were studied at the air-water interface. In the absence of lipid both gramicidins are aligned parallel to the air-water interface and their molecular area (220-230 Å) is compatible with either a single stranded $\pi^{6.3}_{L,D}$ helix or a double helix. In the presence of lipid (dioleoylphosphatidylcholine), the orientation of the gramicidins depends on the pressure of the monolayer: the helical axis is parallel to the interface at low pressure (liquid expanded state) and perpendicular to the interface when the pressure is increased to the liquid condensed state, which is similar to the state of a bilayer. In this latter condition, the molecular area (190 Å) is that expected for the $\pi^{6.3}_{L,D}$ helix. The force-area curves also reveal that whatever the orientation at the peptide is, both gramicidins and the lipid are not miscible and that GA forms larger aggregates than GM. Surface potential measurements show that the surface potential of the mixed monolayers is higher for GA than for GM. This observation is in agreement with the single channel behavior of these two gramicidins. Indeed the conductance of the GA channel is almost independent of the applied voltage, while that of GM strongly depends on the transmembrane potential.

M-Pos333 pH TRIGGERED INTERACTION OF GALA, A SYNTHETIC AMPHIPATHIC PEPTIDE, WITH LARGE UNILAMELLAR VESICLES. Roberta A. Parente, Nanda Subbarao, Laszlo Nadasdi*, & Francis C. Szoka, Jr., Dept. of Pharmacy and Pharmaceutical Chemistry & *Brain Tumor Research Center, Univ. of Calif., San Francisco, CA 94143.

A 30 amino acid peptide containing the repeat unit Glu-Ala-Leu-Ala (GALA) and an N-terminal tryptophan, undergoes a coil to α-helical conformational transition when the pH is reduced from 7.5 to 4.5. The extent of helix correlates with the ability of the peptide to induce leakage of aqueous contents from egg phosphatidylcholine large, unilamellar vesicles. As the pH is lowered from 7.5 to 5.0, the tryptophan undergoes a blue shift in fluorescence. Quenching of tryptophan fluorescence by brominated cholesterol and brominated phosphatidylcholine also occurs at pH 5.0 but not at 7.5. This indicates that the peptide penetrates into the hydrophobic region of the bilayer. The initial rate and extent of leakage of encapsulated contents is dependent on pH, lipid concentration, peptide concentration, and the total concentration of lipid and peptide held at a constant ratio. Leakage at pH 5.0 is characterized by a rapid initial phase followed by a region of slow change after which the extent reaches a plateau. Peptide-induced release of aqueous contents is an all or none event. At lipid to peptide ratios which did not cause 100% leakage, preincubation of GALA with "empty" vesicles greatly reduced leakage from a subsequent addition of probe containing vesicles. This rules out the possibility that peptide molecules rapidly transfer between vesicles or that a significant fraction of peptide remains free in solution. Finally, GALA does not cause lipid exchange between vesicles. A model for the lytic activity of GALA consistent with these results will be presented. Supported by GM 29514 (FCS) & Damon Runyon-Walter Winchell Cancer Fund Fellowship DRG-907 (RAP).

M-Pos334 FLUORESCENCE QUENCHING OF TRYPTOPHAN AT THE LIPID-PROTEIN INTERFACE. L.R. McLean, K.A. Hagaman, T.J. Owen, & J.L. Krstenansky, Merrell Dow Research Institute, Cincinnati, OH 45215

The environment of the single Trp residue of a model amphipathic helical polypeptide (MAP) with sequence SSADWLKAFYDKVAEKLKEAFSSS (Kanellis et al. (1980), J. Biol. Chem. 255, 11464) has been investigated by fluorescence quenching with the water-soluble, neutral quenchers, acrylamide and succinimide. An Edmundson wheel representation of MAP suggests that Trp is located at the lipid-protein interface. Complexes of MAP and dimyristoylphosphatidylcholine (DMPC) were formed by incubation; gel filtration and dialysis showed no free peptide in the complex. At 26°C, relative to MAP in solution, complexes showed a blue shift in the fluorescence emission maximum of the peptide from 352 to 332 nm, an increase in the fluorescence lifetime (measured by the phase shift method) from 1.88 to 2.82 ns, and an increase in the circular dichroic (CD) molar ellipticity at 208 nm from -9300 to -11900 deg.cm^2/dmol. Fluorescence quenching was measured by steady-state and phase shift measurements; the data were analyzed in terms of a collisional and static component. The rate constant for acrylamide quenching was 4.88×10^{-9} $M^{-1}s^{-1}$ (peptide) and $1.27 \times 10^{-9}M^{-1}s^{-1}$ (complex). The ratio of rate constants for succinimide and acrylamide quenching, $\gamma_{s/a}$, was 0.48 for peptide and 0.17 for complex. The static (exponential) quenching constant, V, decreased from 1.04 in the peptide to 0.53 M^{-1} in the complex with acrylamide quenching. Quenching followed the Arrhenius equation with an activation energy of 7.5 kcal/mole. These data show that the Trp of MAP is located in an hydrophobic environment when associated with DMPC and that the succinimide/acrylamide quenching ratio is useful for probing the environment of Trp in peptides associated with lipid.

M-Pos335 AN ACIDIC AMPHIPATHIC PEPTIDE WHICH BEHAVES LIKE SERUM APOLIPOPROTEINS Nanda K. Subbarao, Christopher Fielding † and Francis C. Szoka, Jr. Departments of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy and †Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

The amphipathic helical theory proposes that the active segment of apolipoproteins tends to be α-helical and in the helical conformation, it has a hydrophobic face and a charged face. The relative importance of the charged residues and the hydrophobic residues of the sequence is still debated. We designed a hydrophobic peptide (GALA) whose sequence is W E A A L A E A L A E A L A E H L A E A L A E A L E A L A A. GALA, in the α-helical form has a hydrophobic face and an apposed hydrophilic face. At pH 7.5, GALA does not interact with egg phosphatidylcholine (EPC) liposomes, but it behaves like serum apolipoproteins in its interaction with dimyristoyl phosphatidylcholine (DMPC). The blue shift of tryptophan fluorescence in the presence of DMPC indicates association of lipid with peptide. Circular dichroism spectra indicate that the peptide acquires helical structure in the bound state. The complex with DMPC can be isolated by gel-permeation chromatography. GALA activates the enzyme Lecithin:cholesterol acyltransferase (LCAT) with 80% of the activity of Apo A1, at the same (1:20) (wt/wt) protein to lipid ratio. GALA differs from Apo A1 in interacting with DMPC and not with EPC. Its sequence differ from other studied model Apo A1 peptides in having no positively charged residues. This character, perhaps, makes GALA inactive towards unsaturated lipids. GALA may provide the basis for a simple model system by which the interactions of charged residues can be studied. This work was supported by NIH grant GM 29514 (F.C.S.).

M-Pos336 THE QUENCHING OF TRYPTOPHAN FLUORESCENCE IN A MODEL PEPTIDE BY BROMOLIPID. Elizabeth J. Bolen and Peter W. Holloway. Dept. of Biochemistry, Univ. of Virginia Sch. of Medicine, Charlottesville, VA 22908.

The quenching of fluorescence by depth dependent probes can be used to determine the depth of a protein in the membrane. Previously we synthesized a series of 1-palmitoyl-2-dibromostearoyl-phosphatidylcholines (BRPCs) with bromine atoms at the 4,5-; 6,7-; 11,12-; and 15,16- positions of the sn-2 acyl chain. The utility of these BRPCs as depth dependent probes has now been tested with a synthetic peptide of known sequence. The peptide: Lys_2-Gly-Leu_8-Trp-Leu_8-Lys-Ala-amide is based on the studies of Davis et al. (Biochemistry 24, 1377, 1985). The hydrophobic core of this peptide was designed to match the hydrophobic thickness of the bilayer. When the peptide assumes a trans-bilayer alpha-helical configuration the tryptophan should be found located in the center of the bilayer. The peptide was colyophilized with either 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) or one of the BRPCs and small unilamellar vesicles were prepared by sonication. The binding of the peptide to the vesicles was verified by ultracentrafugational flotation in a sucrose gradient. The fluorescence of each of the peptide-lipid complexes was measured and normalized for the peptide concentration. The fluorescence relative to that in POPC was 87%, 62%, 15%, and 10% for the 4,5-; 6,7-; 11,12-; and 15,16 BRPC respectively, thus confirming the positioning of the tryptophan in the center of the bilayer. These data demonstrate the utility of the bromolipids as depth dependent probes and further validate previous studies done to determine the depth of the fluorescent tryptophan of cytochrome b_5 in membranes. Supported by NIH GM 23858 and a grant from the American Heart Association, Virginia Affiliate.

M-Pos337 THE HYDROPHILICITY OF POLAR AMINO ACID SIDE CHAIN ANALOGS IS MARKEDLY REDUCED WHEN THEY ARE ATTACHED TO A PEPTIDE BACKBONE. Mark A. Roseman, Dept. of Biochemistry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814-4779. Amino acid side chain hydrophobicity scales that are intended to describe the thermodynamics of membrane-protein interactions should be based upon water/organic solvent partition data. Several scales have been proposed that utilize either free amino acids themselves or simple side chain analogs as model compounds. It has always been assumed that the structure-additivity rule applies, i.e., that ΔG_{tr} for each side chain in the model compounds is the same as it is in a polypeptide. However, Yunger and Cramer [Mol. Pharmacol. 20, 602 (1981)] have shown that free amino acids are poor model compounds because the zwitterionic glycyl moiety and the polar side chains self-solvate each other. In the present study the validity of scales based upon ΔG_{tr} of simple side chain analogs was examined by comparing a theoretical scale based on the additivity rule, with the experimental scale of Fauchere and Pliska [Eur. J. Med. Chem. 18, 369 (1983)]. These authors measured the water/octanol partition coefficients of N-acetyl amino acid amide derivatives, a class of compounds in which the side chains are flanked by amide bonds as they are in a polypeptide. The results show that the flanking amide bonds reduce the unfavorable ΔG_{tr}'s for the polar functional groups of the side chains by 35-80%. Similarly, ΔG_{tr} of the backbone amide groups is reduced by about half. These reductions in ΔG_{tr} appear to be due to the "proximity effect" that has been observed with other polyfunctional compounds [Hansch and Leo in Subst. Const. for Corr. Anal. in Chem. and Biol. (Wiley, NY 1979)]. Consequently, the spontaneous insertion of proteins into membranes may be far easier than previously has been thought. A new hydropathy scale based on ΔG_{tr} from water to alkane solvents will be presented. Supported by NIH grant AM30432.

M-Pos338 A COMBINED GENETIC AND BIOPHYSICAL APPROACH TO PROTEIN EXPORT USING TRYPTOPHAN CONTAINING SIGNAL SEQUENCES. M. Rafalski[1], D. Jackson[2], C.J. McKnight[1], M.S. Briggs[1], L.M. Gierasch[1], and T.J. Silhavy[2]. [1]Department of Chemistry, University of Delaware, Newark, Delaware 19716; [2]Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544.

The problem of selective and efficient translocation of proteins across membranes is as yet poorly understood. The most universal requirement for this process is a 15-30 residue long hydrophobic N-terminal extension, termed the signal sequence, that occurs on nearly all secreted proteins. In previous work we demonstrated a correlation of tendency to adopt a helical conformation and of affinity for a lipid membrane with function in signal peptides. [Briggs and Gierasch, Biochemistry 23: 3111, 1984, and Briggs, et.al., Science 228:1096, 1985] We have now incorporated a fluorescent amino acid, tryptophan, in the LamB signal sequence of E. coli as a probe for environment and conformation. Complementary in vivo experiments have introduced the Trp residue by site-specific mutagenesis to assess the impact of the probe on the function of the signal sequence. The first such "biophysical mutant", in which Met 24 has been substituted by Trp, displays a near wild type phenotype in vivo. The isolated Trp 24 signal peptide also behaves like the wild type peptide. By coupling a dansyl fluorophore to the N-terminus of the Trp 24 peptide, we have been able to follow conformational transitions by energy transfer efficiency measurements. The doubly-fluorescently labeled signal peptide adopts predominantly beta structure in aqueous environments and undergoes a transition to alpha helical structure in non-polar environments such as sodium dodecyl sulfate micelles. The interactions of this peptide with lipid vesicles are being used to deduce the rate and topology of signal peptide insertion into lipid membranes.

M-Pos339 CALORIMETRIC AND FLUORESCENCE CHARACTERIZATION OF INTERACTIONS BETWEEN pOTC LEADER PEPTIDE AND PHOSPHOLIPID BILAYERS. Melanie Myers and Ernesto Freire. Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

The interactions of the targeting sequence of the mitochondrial enzyme ornithine transcarbamilase (OTC) with phospholipid bilayers of different molecular compositions have been studied by high sensitivity differential scanning calorimetry, steady state fluorescence anisotropy and excimer formation techniques. These studies indicate that the leader peptide interacts strongly with mixed phosphatidylcholine/phosphatidylserine vesicles but not with pure phosphatidylcholine vesicles. The steady state anisotropy of the fluorescent probe DPH is unaffected when the peptide is added to pure DPPC vesicles; however, the DPH anisotropy shows a large increase in the gel phase of DPPC vesicles containing brain PS at molar ratios as low as 12 percent. Experiments using pyrene-PC indicate a decrease in the rate of excimer formation when the leader peptide is added to vesicles containing phosphatidylserine, suggesting that the leader peptide has a rigidifying effect on the bilayer. The addition of the leader peptide to DPPC:PS (5:1) sonicated vesicles of 200 A radius at a molar ratio of 1/200 results in vesicle fusion. The fusion process is maximal at the phase transition temperature of the vesicles and minimal at temperatures below the phase transition . Below Tm (20 deg C) the fusion process results in vesicles of 360 A radius compared to 1200 A radius at Tm (37 deg C) and 760 A radius at 52 deg C. (Supported by NIH grant NS-24520).

M-Pos340 CRYSTALLIZATION OF MACROMOLECULAR COMPLEXES ON LIPID LAYERS. Hans O. Ribi and Roger D. Kornberg, Department of Cell Biology, Stanford University, Stanford, California 94305.

Macromolecules specifically bound to lipid-linked ligands in planar lipid layers at the air/water interface form two-dimensional crystals suitable for structure determination by electron crystallography. Studies with three proteins and synthetic or naturally occurring lipid-ligands have given well ordered crystals in all cases and have revealed some requirements for this approach. Monoclonal antibodies formed hexagonal lattices on pure lipid-hapten layers under a variety of solution conditions. Crystallization depended on lipid fluidity, and required a high concentration of lipid hapten. Dilution with other phospholipids (filler lipids) gave only parallel rows of antibody lipid complexes. The B1 dimer of E. coli ribonucleotide reductase bound to the lipid-linked effector dATP-capryl-phosphatidylethanolamine (dATP-CAP-PE), formed a rectangular lattice. Imaging at 18Å resolution revealed two domains per B1 monomer. B1 crystallization was optimal on lipid layers with 10% dATP-CAP-PE and 90% egg phosphatidylcholine (PC). Replacing PC with other filler lipids significantly reduced ordering, while addition of multivalent cations to the solution dramatically enhanced it. Cholera toxin, its activated form and the isolated B subunit, all formed isomorphous rectangular lattices on phospholipid or fatty acid layers doped with GM_1. Diffraction extended in each case to about 15Å resolution. Crystallization occurred with a variety of lipid and solution compositions. Three-dimensional structure determination revealed a pentameric ring of B subunits lying flat on the membrane surface with two-thirds of the enzymatically active A subunit penetrating the membrane's hydrophobic interior. Toxin activation caused the catalytic domain to dissociate from the B-oligomer and futher penetrate into the membrane.

M-Pos341 DENATURATION OF THE FRAGMENT 1 DOMAIN IS ALTERED BY BINDING OF PROTHROMBIN TO MEMBRANES. B.R. Lentz, J.N. Carleton, D.R. Alford, P.Berkowitz, and R.G. Hiskey. Depts. of Biochemistry and Chemistry, University of North Carolina, Chapel Hill, NC 27514.

High sensitivity, differential, scanning calorimetry has been used to monitor the effect on prothrombin conformational state of Ca^{2+}-mediated binding of bovine and human prothrombin to large unilamellar vesicles composed of phosphatidylserine/phosphatidylcholine (25/75) mixtures. The thermal denaturation of Ca^{2+} - prothrombin (bovine) was detected calorimetrically as a pronounced endothermic peak at ~ 56°C (2.2 ± .3 cal/g), corresponding to denaturation of the prethrombin 1 portion of the molecule; and a weak endothermic shoulder or peak at ~ 65-68°C (0.6 ± .2 cal/g), corresponding to melting of the fragment 1 domain. The fragment 1 endotherm was substantially reduced and nearly eliminated in the absence of Ca^{2+} (<0.2 cal/g), presumably reflecting the well known Ca^{2+}-induced conformational shift in this domain. Melting of the fragment 1 domain was not detected with human prothrombin in the presence or absence of Ca^{2+}, illustrating the importance of proline isomerization in production of a low-enthalpy, Ca^{2+}-bound state. At concentrations of PS/PC vesicles sufficient (~ 10 mM) to bind 90-95% of available prothrombin (3.6 mg/mL), the peak associated with the fragment 1 domain was broadened, shifted to higher temperature (69-71 C), and enhanced considerably in enthalpy (2 ± .3 cal/g). The melting of the prethrombin 1 domain of prothrombin was at slightly lower temperature (54-55°C) and slightly less enthalpic (1.5 ± .2 cal/g) than in the presence of Ca^{2+} alone. With isolated fragment 1, the denaturation endotherm peaked at 70-72°C and displayed an enthalpy of 1 ± .6 cal/g. The results suggest that binding to lipid may involve conformational communication between the prethrombin 1 and fragment 1 domains.

M-Pos342 SPECTRAL AND STRUCTURAL STUDIES OF SOYBEAN LIPOXYGENASE. J. Andre, R. Carroll, J. Draheim, and M. Funk. U. of Toledo, Toledo, Ohio.

Lipoxygenase (LOX) is a non-heme iron dioxygenase which catalyzes the incorporation of molecular oxygen into polyunsaturated fatty acids containing a 1,4-cis,cis-pentadiene system to yield the 1,3-conjugated hydroperoxy fatty acid. It consists of a single polypeptide chain of molecular weight ca 95,000. Native LOX(FeII) is catalytically inactive. Treatment of LOX with equilmolar hydroperoxy product activates the enzyme to its LOX(FeIII) form. LOX isolated from Provar and Vickery soybean cultivars can be separated into the isoenzymes P1, P4, V1, and V2 by chromatofocusing. P1 and V1 are maximally active at pH9 while V2 and P4 are maximally active at pH7. Fresh native LOX(FeII) exhibits an apparent molar extinction at 280nm of 116,000 (P1); 124,000 (P4); 116,000 (V1); and 119,000 (V2). Upon activation of LOX with equilmolar hydroperoxy product there are minor spectroscopic changes in its near-UV absorption spectrum. These spectral changes could be explained as due to environmental changes of a single Tyr residue. Activation does not alter the shape of the fluorescence emission spectrum for any isoenzyme studied. However, there is a decrease in peak fluorescence at 332nm of 20% (P1); 19% (P4); 25% (V1); and 25% (V2). The results of N-terminus and C-terminus HPLC experiments will also be presented.

M-Pos343 SYNEXIN BINDING TO PHOSPHOLIPID VESICLES. Paul Meers, Demetrios Papahadjopoulos and Keelung Hong, Cancer Research Institute, University of California, San Francisco, CA 94143, U.S.A.

Synexin is a 47 kilodalton cytosolic protein which binds to membranes, aggregates vesicles and enhances fusion rates of liposomes in a Ca^{2+}-dependent manner. The Ca^{2+}-dependent binding of synexin to liposomes composed of phosphatidate:phosphatidylethanolamine (PA:PE) 1:3 could be detected by the fluorescence of membrane-bound probes, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) phosphatidylethanolamine (NBD-PE) and chain labelled pyrene-phosphatidylcholine. The fluorescence intensity of either probe increased upon the addition of Ca^{2+}, only when synexin was also present. These small increases in probe fluorescence were due to a relief of self-quenching or a drop in the efficiency of resonance energy transfer to an acceptor probe and occurred either by the insertion of the protein into the membrane and consequent dilution of the probes or by direct binding of synexin to the probe "protecting" it from quenching. Specific synexin binding to a given probe is regarded as unlikely because similar effects of synexin binding were observed with both probes. Binding of synexin to pure PA and pure phosphatidylserine (PS) liposomes was also detected with the pyrene probe, but no binding to phosphatidylcholine liposomes was observed. Other proteins did not exhibit the Ca^{2+}-dependent effects of synexin. When pyrene-phosphatidylcholine was used as a probe, the ratio of monomer to excimer fluorescence also increased with synexin binding to PA:PE. When excitation was set at 280 nm, synexin binding gave an small increase in probe fluorescence for both the NBD and pyrene probes consistent with a small amount of energy transfer from tryptophan to the probes as acceptors. Acrylamide quenching of tryptophan fluorescence was used to investigate the exposure of tryptophans to solvent when synexin was either free or bound to the membrane. The high efficiency of acrylamide quenching and the relatively high emission wavelength of the tryptophan fluorescence suggest that most tryptophans are highly exposed to solvent in free synexin. Ca^{2+}-dependent binding of synexin to PA:PE liposome membranes produced only a very slight decrease in the average exposure of the tryptophans to acrylamide and no apparent change in the emission maximum. These preliminary data are consistent with some penetration of synexin into the membrane in the presence of Ca^{2+}, while most of its tryptophans remain exposed to the solvent.

M-Pos344 THE LAMELLAR PHASE OF CARDIOLIPIN IS STABILIZED BY THE TRANSBILAYER PROTEIN CYTOCHROME C OXIDASE. Gary L. Powell & Derek Marsh, Department of Biological Sciences, Clemson University, Clemson, SC 29634-1903 and the Max-Planck Institute for Biophysical Chemistry, D-3400 Gottingen, W. Germany.
Cardiolipin isolated from beef heart undergoes an abrupt phase transition with increasing concentrations of NaCl from lamellar to hexagonal (II) phase (Powell and Marsh, Biochemistry 24 (1985) 2902). This phase transition has now been studied in cardiolipin membranes containing the intrinsic transmembranous protein cytochrome c oxidase (beef heart) at various lipid to protein ratios. Hexagonal (II) phase cardiolipin was distinguished by the positive and smaller chemical shift anisotropy using ^{31}P-nuclear magnetic resonance (NMR) from lamellar phase cardiolipin . At NaCl concentrations less than 1.5 M the spectra were for lamellar phase. At higher salt concentrations the spectra appeared composite up to about 4M NaCl when the spectra showed mostly the chemical anisotropy expected for hexagonal (II) phase cardiolipin. The existence of composite spectra over a broad range of salt concentrations contrasted with the abrupt transition observed for pure cardiolipin at concentrations near 1.5 M NaCl, suggesting that the presence of the intrinsic membrane protein stabilized the lamellar phase relative to hexagonal (II) phase of salt concentrations exceeding 1.5 M. In 4 M salt, increasing the relative concentraton of protein increased the proportion of lamellar phase; when the proportion of lipid was increased, the fraction of hexagonal (II) phase increased. These observations again suggest that the protein stabilized the lamellar phase. (Supported in part by Grant-In-Aid #82952 from the American Heart Association).

M-Pos345 EFFECTS OF STEROLS ON THE STRUCTURE AND FUNCTION OF THE ACETYLCHOLINE RECEPTOR.

Julie P. Earnest, Michael J. Shuster, and Robert M. Stroud. Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, CA 94143.

The nicotinic acetylcholine receptor (AChR) has been successfully reconstituted into lipid vesicles containing a mixture of zwitterionic phospholipids, negatively charged phospholipids, and cholesterol. Experiments in our lab examining the morphology and function of reconstituted vesicles using various lipid mixtures, and with several different reconstitution protocols, have established that although cholesterol plays an important role in lipid vesicle stability, cholesterol also has dramatic and specific effects on AChR function (Earnest et al., Membrane Proteins, S. Goheen et al., Eds., in press.) Recent FTIR experiments confirm that cholesterol is required to maintain the AChR secondary structure necessary for agonist-induced channel opening (Fong and McNamee, Biochemistry, in press.) Our current experiments involve biochemical and structural characterization of the specific sterol-receptor interactions. Preliminary data suggest that the sterol detergent sodium cholate occupies the same AChR binding sites as cholesterol. Displacement of bound cholesterol by sodium cholate may be involved in the lipid-dependent detergent-inactivation which is observed in AChR reconstitution. A structural model for sterol-AChR interaction is proposed.

M-Pos346 STABILIZATION OF ACETYLCHOLINE RECEPTOR SECONDARY STRUCTURE BY SPECIFIC LIPIDS, Tung Ming Fong, Anil Bhushan, & Mark G. McNamee, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

Fourier transform infrared (FTIR) spectroscopy was used to study the secondary structure of purified Torpedo californica nicotinic acetylcholine receptor (AChR) in reconstituted membranes. Functional studies have previously demonstrated that the ion channel activity requires the presence of both sterol and negatively charged phospholipids in membranes. The present studies are designed to test the hypothesis that the α-helical structure of AChR may be stabilized by sterol and/or negatively charged phospholipids, and that these α-helices are involved in the formation of a potential ion channel. FTIR spectra of peptide backbone vibration have been analyzed to obtain an empirical estimation of secondary structural contents. Our hypothesis is supported by FTIR data which demonstrate that cholesterol molecules in membranes increase the the AChR α-helical content from about 17 % in membranes without sterols to about 20 % in membranes containing sterols ($p < 0.005$). Moreover, negatively charged phospholipids cause an increase in AChR β-sheet content, but not α-helical content, from about 21 % in membranes devoid of negatively charged phospholipids to 25 % in membranes containing negatively charged phospholipids ($p < 0.005$). It is concluded that specific interaction between membrane protein and lipid molecules are required to maintain the secondary structures (α-helices and amphipathic β-sheet) to support the ion channel function of AChR. Further studies of the amide I absorption in D_2O will also be discussed.

M-Pos347 TUBULIN FORMS AN INTEGRAL MEMBRANE SKELETON IN MOLLUSCAN GILL CILIA. R. E. Stephens and S. Oleszko-Szuts, Marine Biological Laboratory, Woods Hole, MA 02543 (Intr. by Shinya Inoué).

Previous work has demonstrated that scallop ciliary membrane tubulin can be reassociated with natural lipids simply by removal of the solubilizing detergent, followed by a freeze-thaw cycle (J. Cell Biol. 96: 68, 1983). Membrane tubulin is associated with numerous minor proteins and with lipids, forming a high molecular weight complex (J. Cell Biol. 100: 1082, 1985). The lipid may be displaced by condensation with Triton X-114 above the cloud point, forming sedimentable lipid-detergent mixed micelles, while the proteins are rendered soluble by complex formation with detergent (Biochim. Biophys. Acta 821: 413, 1985). Controlled extraction of intact gill tissue, isolated cilia, or reconstituted membrane vesicles with Triton X-100 (or NP-40) at concentrations >4 times the cmc (or octyl glucoside at the cmc) delipidates the membrane, leaving a membrane remnant or skeleton of membrane tubulin and associated proteins. This skeleton consists of a disordered reticular protein network in reconstituted membrane vesicles and a similar but more compact sleeve in cilia of extracted tissue. The membrane skeleton is closely apposed to the axoneme and is attached to the outer doublets by fine radial bridges having a 200-240 A longitudinal periodicity, supporting earlier observations made utilizing a lipophilic cross-linking agent (J. Cell Biol. 84: 381, 1980). The reticular material contains tubulin, demonstrated on Lowicryl K4M thin sections by a rabbit polyclonal antibody to sea urchin sperm flagellar tubulins, using gold-labeled second antibody. Minimal cross-reactivity is detected prior to Triton-delipidation, suggesting that most membrane tubulin antigenic sites are buried within the bilayer and that the tubulin is not simply adsorbed to the lipid bilayer. Supported by NIH grants GM 20,644 & GM 29,503.

M-Pos348 ROLE OF LIPID-LIPID INTERACTIONS IN REGULATING THE ADSORPTION OF PANCREATIC CARBOXYLESTER LIPASE TO LIPID-WATER INTERFACES. T. Tsujita and H. L. Brockman, Hormel Institute, University of Minnesota, Austin, Minnesota 55912.

Physical measurements of neutral lipids in mixtures with 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) have revealed the formation of "complexes" or preferred packing arrays [J. M. Smaby and H. L. Brockman, (1985) Biophys. J. 48 701-707]. The marked changes in packing and hydration which were observed suggested that complexation might regulate the interaction of proteins with mixed lipid surfaces. To explore this, the absorption of pancreatic carboxylester lipase (CEL) to POPC-docosadienoic acid (DDA) films at the Ar-buffer interface was measured. At pH 6.6 adsorption to DDA exhibited saturation behavior with a K_d of 52 nM and maximal adsorption of 3.9 pmol/cm^2. The latter corresponds closely to the formation of a CEL monolayer (~4.1 pmol/cm^2) at the interface. At pH 5.6 K_d was 13 nM indicating a dependence on DDA or CEL ionization state. With POPC-DDA mixtures, CEL adsorption was highly composition dependent. Little or no binding to POPC or POPC-DDA complex (~1:2) was observed, but CEL did bind when uncomplexed DDA was present with complex. CEL binding at 220 nM was a direct function of the proportion of uncomplexed DDA in the surface but not all potentially available DDA surface was covered. Determination of K_d at 0.8 and 0.9 DDA gave values of 669 and 447 nM, suggesting composition dependent changes in DDA ionization or packing. In either case, the data clearly show that lipid-protein interactions are regulated not only by lipid composition but also by lipid-lipid interactions within the surface phase. [Supported by HL17371 and the Hormel Foundation.]

M-Pos349 **ACTIVATION OF PROTEIN KINASE-C BY SHORT CHAIN PHOSPHATIDYLSERINE.** Jeffrey M. Walker and Julianne J. Sando (Intr. by Rodney L. Biltonen), Depts. of Biophysics and Pharmacology, University of Virginia, Charlottesville VA. 22908.

Protein Kinase-C (PKC), like many other Ca^{+2}-dependent membrane-binding proteins, shows specificity for acidic phospholipids, particularly phosphatidylserine (PS). The reason for this specificity has not been established, although surface charge and lipid phase characteristics are commonly suggested. It is also possible that these lipids act as monomeric activators at a hydrophobic region of the protein. To distinguish between these possible mechanisms, the ability of monomeric PS to activate PKC was examined. 1,2-dihexanoyl-phosphatidylserine (PS_6) was prepared from phosphatidylcholine (PC) by a one-step transphosphatidylation catalyzed by phospholipase D in the presence of L-serine. Bovine (mixed long chain) PS provided maximal stimulation of kinase activity at a concentration of 50 to 100 uM PS, 80 uM Ca^{+2}, and 2.6 uM sn-1,2-dioctanoylglycerol (diC_8). Stimulation was minimal in the absence of diC_8. PS_6 had only a minimal stimulatory effect at 50 uM, but stimulation comparable to that with bovine PS was seen at 1000 uM PS_6, a concentration lower then the predicted critical micelle concentration for pure PS_6. Even greater stimulation was seen when the Ca^{+2} level was increased to 300 uM. 80% of maximal PS_6 stimulated kinase could be achieved in the absence of diC_8. The addition of egg PC to either the long or short chain PS significantly reduced the maximal kinase activity. These results suggest that the interaction between PKC, lipid, Ca^{+2}, and diacylglycerol may be more complex then a simple surface phenomenon. Studies are currently being extended to a series of PS chain lengths in order to better correlate enzyme activity to the macromolecular lipid structure. (Supported by NIH Grants GM31184 and T32-GM07267).

M-Pos350 LIPID DOMAIN STRUCTURE CORRELATED WITH MEMBRANE PROTEIN FUNCTION IN PLACENTAL MICROVILLOUS VESICLES. Nicholas P. Illsley, Herbert Y. Lin, and A.S. Verkman. Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

Membrane fluidity properties of placental microvillous membrane vesicles (MVV) were determined from fluorescence anisotropy (r), dynamic depolarization and lifetime heterogeneity studies of diphenylhexatriene (DPH), trimethylamino-DPH (TMA-DPH) and cis- and trans-parinaric acids (c-PnA and t-PnA). Plots of r against temperature for DPH and TMA-DPH in MVV had slope discontinuities at 26°C (T_c, transition temperature), however analysis of r in terms of probe rotational rate (R), limiting anisotropy (r_∞) and lifetime (τ) revealed that DPH reported a phase transition because of changes in r_∞, whereas the phase transition observed by TMA-DPH occurred primarily because of changes in R. Heterogeneity analysis using phase and modulation lifetimes at three frequencies showed that DPH and TMA-DPH lifetimes were homogeneous in MVV. Both long (>25ns) and short (<6ns) lifetime components were detected for c-PnA and t-PnA in MVV, corresponding to the probes in solid and fluid lipid phases. The fractional amplitude of the long lifetimes (solid phase) decreased from 0.86 to 0.12 with increasing temperature (5-55°C) as the membrane passed through the phase transition, with 50% of the change occurring at 27°C (c-PnA) or 33°C (t-PnA). The activation energies for alkaline phosphatase, aminopeptidase M and Na/H antiporter activities all showed discontinuities in the temperature range 27-31°C. These results indicate: (1) time-resolved fluorescence measurements are required to interpret correctly changes in steady-state anisotropy with temperature, (2) both solid and fluid lipid phases coexist in the MVV membrane over a broad temperature range centered around the phase transition temperature, and (3) membrane fluidity has an important modulating influence on microvillous membrane enzyme and transport functions.

M-Pos351 FLUORESCENCE MEASUREMENTS OF COMPLEMENT-MEDIATED BACTERIAL INNER MEMBRANE DAMAGE. John R. Dankert, Department of Comparative and Experimental Pathology, University of Florida, Gainesville, FL 32610.

Studies on the mechanism of action of human complement on Gram-negative bacteria reveal the rapid (<2 min) inhibition of proline transport and the loss of accumulation of membrane potential-sensitive probes, such as tetraphenylphosphonium and methyl thio-β-D-galactopyranoside, in response to complement attack (Dankert & Esser, Biochemistry 25 (1986) 1094). The neutral dye, N-phenyl-1-naphthylamine (NPN) has been characterized as a probe of de-energization of the bacterial inner membrane. An increase in the extent and rate of dye fluorescence has been shown to be proportional with the level of de-energization induced by uncouplers of oxidative phosphorylation or bacterial toxins (Phillips & Cramer, Biochemistry 12 (1973) 1170). Using complement-sensitive strains bearing preformed C5b-8 sites in the presence of NPN addition of C9 leads to an increase in dye fluorescence. This phenomenon occurs in complement-sensitive strains of E. coli and S. typhimurium. When C9 is a added alone to these cells or to lipid vesicles or to complement-resistant strains bearing C5b-8 complexes no increase in fluorescence is seen. The rate of fluorescence change is comparable to the rate of inhibition of proline transport or loss of membrane integrity as measured by other methods. (Supported by NIH Grant R01 AI-22912)

M-Pos352 TRANSFER OF SURFACE PROTEINS FROM MURINE LYMPHOCYTES TO PHOSPHOLIPID VESICLES: INTERMEMBRANE PROTEIN TRANSFER. Alexandra C. Newton & Wray H. Huestis, Department of Chemistry Stanford University, Stanford, CA. 94305

Incubation of cultured murine lymphocytes (BL/VL3) with sonicated dimyristoylphosphatidylcholine (DMPC) vesicles results in the appearance of three lipid species in the supernatant which can be separated by density gradient centrifugation. Analysis of protein and lipid composition, assays for cell and vesicle contents, and proteolytic digestions reveal that the densest species is composed of sealed plasma membrane fragment-vesicle complexes, while the least dense, which comigrates with pure phospholipid vesicles, is unaltered sonicated vesicles. An intermediate density species comprises topologically intact sonicated vesicles with associated plasma membrane proteins. Proteolytic analyses of these protein-vesicle complexes indicate that a 75 kdalton surface iodinated protein inserts in native orientation into the vesicle bilayer, with the cytoplasmic segment contacting the aqueous lumen of the vesicle. Immunological studies reveal that protein-vesicle complexes are highly enriched in the phenotypic marker Ly 1. In contrast, Ly 2, T 200, and Thy 1 do not transfer to the vesicles. Previous work established that functional band 3, the erythrocyte anion transporter, inserts in native orientation into the bilayer of sonicated DMPC vesicles (Newton et al., Biochemistry 22, 6110, 1983, and Huestis and Newton, J. Biol. Chem., in press). The present findings indicate that intermembrane protein transfer is a general phenomenon, and is not restricted to erythrocyte-vesicle interactions.

M-Pos353 **HIGH SENSITIVITY DIFFERENTIAL TITRATION CALORIMETRY OF PROTEIN ASSOCIATION AND INSERTION INTO MEMBRANES.** Obdulio Lopez Mayorga, Jennifer Emtage and Ernesto Freire, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

A newly designed differential titration calorimeter module has been optimized to measure the association and insertion of peptides and proteins into membranes. The differential design of the instrument allows automatic real time correction of heat effects associated with reactant dilution as well as mechanical effects due to injection and continuous stirring of the sample, thus improving the effective sensitivity of the microcalorimeter. Conventional titration calorimeters, including our previous design, require separate experiments to estimate the magnitude of these effects before the data can be corrected. The sensitivity of this instrument is better than 25 ncal/sec thus allowing precise measurements using very dilute samples. We have used this instrument to measure the enthalpy of association of bee venom melittin to large unilamellar DEPC vesicles at very low protein concentrations (50 ug/ml) and very low protein/lipid molar ratios. In the fluid phase of the phospholipid the association is characterized by an exothermic enthalpy of -30 ± 1 kcal/mol of melittin. In conjunction with differential scanning calorimetric experiments, these data suggest that the main contribution to the enthalpy of association arises from the peptide induced perturbation of the physical state of the phospholipid molecules in immediate contact with the melittin molecule. (Supported by NIH grant GM-37911).

M-Pos354 3-D STRUCTURE OF Na,K-ATPase BY ELECTRON MICROSCOPY AND IMAGE RECONSTRUCTION. M. Mohraz and P.R. Smith. Dept. of Cell Biol. New York Univ. Sch. of Med. New York, NY 10016.

Na,K-ATPase constitutes the Na,K pump in the plasma membrane of animal cells. It derives energy from ATP hydrolysis for the transport of Na^+ out of the cell and K^+ into the cell. The enzyme can be purified in membrane-bound form and has been shown to consist of two polypeptide chains: the catalytic subunit α (M_r=110,000) and a glycoprotein β (M_r=50-60,000).

Two-dimensional arrays of the enzyme were induced by partial removal of its phospholipids by phospholipase A_2, and in the presence of various ions (Mohraz et al. 1985. JUR 93:17-26). The arrays were exclusively dimeric with an $(\alpha\beta)_2$ structure in the unit cell. Tilt series were recorded from the negatively stained specimen of the sheets at tilt angles up to 60°. Data from seven tilt series were included in the computation of the three-dimensional structure to a resolution of 2.5nm. The reconstruction shows a large mass on one side of the lipid bilayer, as determined from the side views of the sheets. This structure, which is 6.0nm in depth, is attributed to the segments of the enzyme that protrude from the cytoplasmic side of the membrane. The reconstructed structure consists of ribbons of paired molecules. The most massive region of the protein is tilted with respect to the plane of the membrane. Within the ribbons, the interaction that is responsible for the formation of the dimer bond is clearly visible and is identified as the amino terminal portion of the α subunit. The ribbons are interconnected by bridges that are located close to the membrane. These bridges were tentatively identified in the projection images as the β subunit (Mohraz and Smith. 1984. JCB 98:1836-1841). Since β subunit protrudes only slightly into the cytoplasm, the proximity of these bridges to the membrane further supports this identification. Supported by NIH grants GM-26723 and GM-35399.

M-Pos355 THE ROLES OF CHOLESTEROL AND BUTYLATED HYDROXYTOLUENE IN THE LIPID POLYMORPHISM AND THE Ca-TRANSPORT FUNCTION OF Ca-ATPase by Cheng,K.-H.,Hui,S.W.,Yeagle,P.L.*,Lepock,J.R.†,and K.Jeffrey‡, Biophysics Dept.,Roswell Park Memorial Instit.,Buffalo,*Biochemistry Dept.,SUNY/Buffalo, †Physics Dept.,U.of Waterloo, Canada, and‡Physics Dept.,U. of Guelph,Canada.

The effects of the motional order of fatty acyl chains and the bilayer destabilization characteristics of lipids on the Ca-transport function of reconstituted Ca-ATPase were examined. Lipid mixtures containing different ratios of soybean PE, egg PC, and cholesterol in the presence or absence of lipid perturbant butylated hydroxytoluene (BHT) were studied. At high PE content (>50%), both cholesterol and BHT (23%) effectively promote the hexagonal phase formation of the pure lipid mixtures as determined by the P-31 NMR and freeze-fracture electron microscopy and enhance the Ca-transport function of the reconstituted Ca-ATPase. Yet cholesterol increases and BHT decreases the motional order of the fatty acyl chains of the membranes as determined by the rotational dynamics of the fluorescent probe (DPH) and spin label (2N14). The H-2 NMR study of perdeuterated BHT in either PC or PE mixtures suggested that the BHT molecules are not ordered and most likely are located near the center of the bilayer. Our study indicated that the Ca-transport function of Ca-ATPase depends on the bilayer surface hydration and packing geometry of the lipids but not on the motional order of the lipid fatty acyl chains.

M-Pos356 ^2H NMR OF GRAMICIDIN-A IN AN ORIENTED LYOTROPIC NEMATIC PHASE James H. Davis, (Intr. by F. Ross Hallett), Biophysics Interdepartmental Group, Physics Department, University of Guelph, Guelph Ontario, CANADA N1G 2W1

The ^2H NMR spectra of exchange deuterated gramicidin-A in an oriented lyotropic nematic phase are in agreement with earlier work on unoriented dispersions of gramicidin-A in dipalmitoylphosphatidylcholine [Datema, Pauls and Bloom Biochemistry 25, 3796 (1986)], confirming the notion that oriented micellar phases are useful models of lipid bilayers. In addition, the sharp detail in the spectra of the oriented samples provides a more quantitative picture of the backbone structure of gramicidin-A in a hydrophobic environment. The relaxation behaviour of the eleven clearly resolved doublets observed provides valuable information on the backbone dynamics of the peptide.

M-Pos357 **MEMBRANE FLUIDITY CHANGES AND NEUTROPHIL BACTERICIDAL ACTION.** Jack Blazyk*, Fuz Rana*, Michael Loeffelholz and Malcolm Modrzakowski, *Chemistry Dept. & College of Osteopathic Medicine, Ohio University, Athens, OH 45701 (Intr. by Paul Sullivan)

Rat neutrophils, which protect against infection in the blood, produce several small cationic peptides possessing bactericidal activity in vitro. The Gram-negative bacterium, Acinetobacter calcoaceticus, is susceptible to these peptides. This organism can acquire the resistance plasmid RP1, which increases both the permeability of the outer membrane and its sensitivity to cationic peptides. In addition, growth on defined hydrocarbon media, which significantly alters the fatty acyl composition of the membrane lipids, modulates the bactericidal effect of the peptides. The fluidity of the outer membrane has been implicated as a potential factor in determining the bactericidal potency of the cationic peptides. We have used FT-IR spectroscopy to measure temperature-dependent changes in the frequency, relative area and bandwidth of the symmetric methylene stretching band of the lipid hydrocarbon chains in inner and outer membranes. Thermotropic phase changes between 20 and 25°C were detected in inner and outer membranes of organisms grown on soy broth at 37°C. As a first step in testing whether membrane fluidity indeed correlates with the killing process, the effects of RP1 acquisition and growth on defined hydrocarbons upon thermotropic phase changes in the lipids of inner and outer membranes were studied. In $RP1^+$ A. calcoaceticus, no change was seen in the fluidity of the inner membrane as compared to that of the plasmid-free organism; however, outer membrane fluidity is decreased significantly. Data showing the consequences of altering the hydrocarbon growth supplement in both $RP1^-$ and $RP1^+$ organisms are presented and the relationship between membrane fluidity and susceptibility to cationic peptides is discussed.

M-Pos358 DPH FLUORESCENCE LIFETIME DISTRIBUTION IN ERYTHROCYTE MEMBRANES. R. Fiorini (*), G. Curatola (*), E. Gratton (**). (*)Biochemistry Institute, University of Ancona, ITALY. (**) Department of Physics, University of Illinois, Urbana, IL.

Multifrequency phase fluorimetry is a useful tool to study membrane heterogeneity. By using this technique, the fluorescence lifetime of 1,6-diphenyl-1,3,5-hexatriene(DPH) in erythrocyte membranes obtained from normal and cholesterol depleted erythrocytes was analysed with a continous distribution of lifetimes values. In all cases a simple lifetime distribution accounts for at least 95% of the observed decay. In normal erythrocyte membranes this main component has a center lifetime value of about 11.2 nsec at 4°C which slightly decreases increasing the temperature, while it shows a narrow distribution width (0.05 nsec) at all temperature range. On the contrary in cholesterol depleted erythrocyte membranes the distribution shows a broader width (about 1 nsec) whereas the central value has almost the same pattern which was observed in the control. Our results show that the degree of heterogeneity in erythrocyte membranes is related to the membrane phospholipid/ cholesterol ratio. This interpretation is confirmed by the observation that in egg lecithin multilamellar liposomes the presence of cholesterol strongly affects the DPH lifetime distribution width. We suggest that DPH lifetime distributional analysis can be used to study physical and physico-chemical aspects of natural membrane heterogeneity. Support by Regione Marche - Ricerca Sanitaria Finalizzata 213, 22/5/85.

M-Pos359 NMR STUDIES OF PHORBOL ESTER AND DIGLYCERIDE EFFECTS ON PHOSPHOLIPID STRUCTURE. B.A. Lewis and J.S. Cook, Dept. of Chemistry, University of Wisconsin, Madison WI 53706 and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

Diglycerides (DGs) have been shown to perturb the structure of phospholipid bilayers, inducing the formation of bulk non-bilayer phases such as hexagonal (H_{II}) when present at sufficiently high concentrations (Das and Rand (1984) Biochem. Biophys. Res. Commun 124: 491; Das and Rand (1986) Biochem. 25: 2882; Dawson et al. (1984) Biochem. Biophys. Res. Commun. 125: 836). Transient production of small amounts of DGs by phospholipase activity in vivo has significant physiological effects, including the activation of protein kinase C (PKC). Another class of PKC activators are the tumor-promoting phorbol esters such as 12-O-tetradecanoyl phorbol-13-acetate (TPA), and it has been proposed that TPA mimics DG in its physiological effects. It is thus of interest to compare the effects of TPA on the structure of phospholipids with those of DGs, and ^{31}P NMR is an ideal tool for this comparison. Our preliminary results suggest that TPA does not mimic DG in its effects on phospholipid structure, at least at the level of induction of bulk transitions from lamellar to hexagonal phases. More subtle effects of DGs on phospholipid structure have been observed in PC-PS mixtures as a function of Ca^{++} concentration, and these effects are compared with those of TPA. (Supported by NCI and by OHER, USDOE under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.).

M-Pos360 COMPARISON OF THE EFFECT OF LANOSTEROL AND CHOLESTEROL ON MYCOPLASMA CAPRICOLUM CELL MEMBRANE STRUCTURE AND LIPID COMPOSITION. Tai-Huang Huang, School of Physics, Georgia Institute of Technology, Atlanta, GA 30332; August J. DeSiervo and Alma D. Homola, Department of Microbiology, University of Maine, Orono ME 04469

M. Capricolum (ATCC 27342, California Kid Strain 14) were grown in modified Edward medium supplemented with defined fatty acids. Cells grown in various amount of cholesterol and Lanosterol were analyzed for their lipid compositions by 2-D paper chromatography and showed that the predominant species being Phosphatidylglycerol (PG) (60%) and Cardiolipin (CL) (35%). The amount of cholesterol in the growth medium had little effect on the composition. The fatty acid composition, analyzed by gas-liquid chromatography showed an preferential enhancement of incorporation of oleic acid into cell membrane lipids at higher amount of cholesterol. The oleic acid to palmitic acid molar ratio changed from 0.3 at 1.25 ug/ml cholesterol to 0.5 at 20 ug/ml cholesterol. The cholesterol/phospholipid molar ratio increases nonlinearly, suggesting a controlled, not passive incorporation of cholesterol and lipids. Lanosterol showed similar effects observed with cholesterol. We have also incorporate (7,7,8,8-d4) palmitic acid into cell membrane. ^{2}H NMR showed that although at the growth temperature (37c) cell membranes were in liquid crystalline state in both conditions (20 ug/ml cholesterol or 20 ug/ml lanosterol) ^{2}H NMR spectra obtained at lower temperatures indicates that cells grown in lanosterol appeared to have more rigid membranes, comparable to that of cells grown in lower cholesterol concentration (1.25ug/ml). Therefore lanosterol appears to be less effective in modulating membrane fluidity.

M-Pos361 WARMING INDUCES INTRACELLULAR FREEZING IN FROZEN TENDER POPULUS Allen Hirsh and Robert J. Williams, American Red Cross, 9312 Old Georgetown Road, Bethesda, Maryland 20814 and Eric Erbe, Electron Microscopy Laboratory, USDA, Beltsville, Maryland 20874

Using freeze-fracture, freeze-etch electron microscopy we have examined the effects of partial thawing on frozen tender Populus. The tissue was prepared using very slow rates of cooling and warming (< 6°C/hr). Our results are not consistent with the hypotheses that cells adhere to extracellular ice (Olien and Smith(1)), or that the observed damage is caused by plasma membranes bursting upon re-expansion during warming, with subsequent invasion by extracellular ice (Gordon-Kamm and Steponkus (2)).

We found that tissue warmed even a few degrees C during a cooling regimen showed extensive intracellular freezing but displayed evidence of the freezing only in the inner leaflet of the plasma membrane. We postulate that injury to plasma membranes during cooling and subsequent rewarming leads to the seeding of partially supercooled intracellular solutions during the warming phase. Thus intracellular freezing during warming may be a significant cause of freezing injury in woody tissue.

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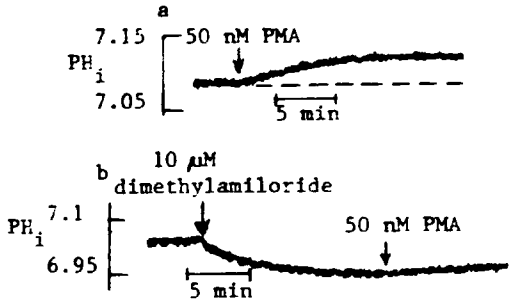
(2) Gordon-Kamm, W.J. and Steponkus, P.L. "The Behavior of the Plasma Membrane Following Osmotic Contraction of Isolated Protoplasts: Implications in Freezing Injury" Protoplasma 123, 83-94 (1984)

Supported by NIH grants BSRG2S07 RR05737 and GM 17959.

M-Pos362 PHORBOL ESTER AND DIACYLGLYCEROL ACTIVATE Na-H EXCHANGE IN RAT VENTRICULAR MYOCYTES. By V. K. Sharma and S-S. Sheu, Department of Pharmacology, University of Rochester, Rochester, NY 14642.

There is now good evidence that Na-H exchange may have an important role in the regulation of intracellular H^+ concentration (PHi) in cardiac muscle cells (Deitmer and Ellis, J. Physiol. 304: 471, 1980; Piwnica-Worms et al., J. Gen. Physiol. 85:43, 1985). Using the fluorescent indicator 2',7'-bis (carboxyethyl)-5,6-carboxyfluorescein (BCECF), we investigated the effect of phorbol ester and diacylglycerol on PHi in suspensions of rat ventricular myocytes. As indicated in the figure, addition of 50 nM phorbol 12-myristate 13-acetate (PMA) to a suspension of myocytes caused a small increase in PHi. This increase was also produced by synthetic diacylglycerol 1-oleoyl-2-acetyl-glycerol (OAG, 20 μM). This PMA-induced PHi change was mediated via the activation of Na-H exchange since prior exposure of the cells to 10 μM dimethylamiloride, a specific blocker of Na-H exchange, prevented the change. Similarly, in Na_o-free solution, 50 nM PMA produced no change in PHi (record not shown). Since phorbol ester and diacylglycerol are potent activators of protein kinase C (PKC), it seems likely that the observed PHi changes are the result of Na-H exchange activation via PKC.



M-Pos363 DIRECT EVIDENCE FOR NA^+-CA^{2+} EXCHANGE AND CA^{2+} PUMP IN SMOOTH MUSCLE CELLS. Hiroshi Yamaguchi (Intr. by J. Dobson), Dept. of Physiology, University of Massachusetts Med. School, Worcester, Ma 01605.

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in smooth muscles is likely maintained by two major Ca^{2+} extrusion mechanisms; one coupled with the Na^+ influx (Na^+-Ca^{2+} exchange) and the other a metabolically driven Ca^{2+}-pump. However, neither mechanism has been demonstrated by direct, intracellular Ca^{2+} probes and thus, these Ca^{2+} extrusion mechanisms remain largely speculative. To identify these mechanisms concentric double-barreled Ca^{2+} electrodes with tip size, ~0.1 μm (Cell Calcium 7;203-219, 1986) were employed to record simultaneously changes in $[Ca^{2+}]_i$ and in the resting membrane potential (E_m) in single smooth muscle cells enzymatically dispersed from toad stomach. Two extrusion mechanisms were operationally defined as the one sensitive to removal of external Na^+ (Na^+-Ca^{2+} exchange) and the other sensitive to the calmodulin antagonist, calmidazolium (Ca^{2+} pump). When Na^+ was replaced with Li^+, a rapid rise in $[Ca^{2+}]_i$ ($t_{1/2}$ < 30sec) was invariably seen followed by a plateau phase with a gradual Ca^{2+} elevation. The initial elevation of Ca^{2+} averaged 780 ±110 nM (SEM, n=5). Calmidazolium at concentrations greater than 5μM applied during a plateau phase (n=3) caused additional rise of $[Ca^{2+}]_i$ at a rate of about 150 nM/min. Calmidazolium in normal physiological salt solution also caused a rise of $[Ca^{2+}]_i$. These results suggested that in toad visceral smooth muscles both the Na^+-Ca^{2+} exchange and the Ca^{2+} pump play a significant role in maintaining low $[Ca^{2+}]_i$. (supported by a grant from PHS HL35807)

M-Pos364 PARTIAL PURIFICATION OF THE Na/Ca EXCHANGER FROM CANINE CARDIAC SARCOLEMMA. Kenneth D. Philipson and Robert Ward. Departments of Medicine and Physiology and the Cardiovascular Research Laboratories. UCLA School of Medicine, L.A., CA 90024

We have begun studies to purify the Na/Ca exchange protein from canine cardiac sarcolemmal (SL) vesicles and have developed techniques useful towards this goal. Alkaline extraction at pH $\geq$ 12 removes specific proteins from SL. Vesicle permeability barriers are destroyed but complete retention of Na/Ca exchange activity is evident after solubilization and reconstitution. About 50% of protein is removed, and exchanger specific activity is doubled. Alkaline extraction probably removes peripheral proteins as is the case with red cell membranes. We have also examined the interaction of solubilized exchanger with wheat germ agglutinin (WGA)-agarose columns. In Triton X-100 (1.5%)/asolectin (1.0%) only about 20% of exchange activity specifically binds, whereas in Chaps (8 mM) detergent without asolectin about 75% of exchanger activity binds to WGA. Binding of most other proteins is unaffected by conditions. By using two WGA columns in series, first in Chaps and then in Triton/asolectin, we are able to enrich Na/Ca exchange activity. SDS-PAGE suggests that the exchanger may be 33 kDa, as inferred by Soldati et al (J. Biol. Chem. 260:13321, 1985), or a minor component of the SL.

M-Pos365 Na/Ca EXCHANGE IN CARDIAC SARCOLEMMA (SL) VERSUS CALCIUM: EFFECTS OF MEMBRANE POTENTIAL. R.H. Smith and G.E. Lindenmayer. Depts. Pharmacology & Medicine, Medical University of South Carolina, Charleston, SC 29425

A previous study (J.Memb.Biol. 84:207,1985) suggested that Na/Ca exchange in SL vesicles may be qualitatively different below and above 0 mV, viz., under near Vmax conditions for outside Ca, the rate nearly doubled between -100 and 0 mV but doubled again between 0 and +30 mV. The study below probes the nature of this change. SL was loaded with either 100 mM NaCl or LiCl, 60 mM KCl, 20 mM MOPS/Tris (pH 7.4 for 37°), 1 mM $MgCl_2$, 5 mM EGTA/Tris and 1 µM valinomycin. The loaded SL was subjected to 5 freeze-thaw cycles to insure equilibration of EGTA across the membrane. Assays (1 or 3 sec) for Na-dependent Ca uptake were initiated by a 1:20 dilution into media at 37° containing 5 mM NaCl or LiCl, varying KCl and 1 µM valinomycin (to yield Nernst potentials, E_K) and choline Cl (to keep ionic strength constant), 20 mM MOPS/Tris (pH 7.4), 1 mM $MgCl_2$, 1 mM EGTA/Tris and $CaCl_2$ with ^{45}Ca to yield desired free Ca (verified by electrode). Initial rates of Na-dependent Ca uptake were measured over free Ca ranges of 0.2 to 120 µM for E_K = -80 (inside negative) and 0 mV; 0.1 to 102 µM for E_K = +26 mV. Fit of the data to the Hill equation yielded: Vmax = 0.96, 1.55 and 3.19 nmol/mg/sec; $K_{0.5}$ = 2.1, 2.8 and 12.1 µM; Hill coefficient = 1.66, 1.87 and 1.06 for E_K = -80, 0 and +26 mV, respectively. Conclusions: (1) membrane potential has disproportionate effect on Vmax below and above 0 mV; (2) this is accompanied by a small increase (33%) in $K_{0.5}$ from -80 to 0 mV but a 4.3-fold increase from 0 to +26 mV; (3) at 0 mV and below, more than one calcium site may be involved in the exchange reaction; at +26 mV only one site appears to be involved.

M-Pos366 DEPENDENCE OF Na^+/Ca^{2+} EXCHANGE ACTIVITY IN RECONSTITUTED SARCOLEMMAL VESICLES ON PHOSPHOLIPID COMPOSITION. Ramesh Vemuri and Kenneth D. Philipson (Intr. by J.S. Frank). Depts. of Medicine and Physiology, Cardiovasc. Lab., UCLA School of Medicine, L.A., CA 90024.

We solubilized and reconstituted the sarcolemmal Na^+/Ca^{2+} exchanger using Triton X-100 and either asolectin (soy bean phospholipids) or various combinations of phospholipids (PL). About 3-5 fold stimulation of Na^+/Ca^{2+} exchange over native sarcolemmal vesicles was obtained using asolectin (V_{max} of about 110 nmol $mg^{-1}sec^{-1}$). Reconstitution using various combinations of phosphotidylcholine (PC), phosphatidylethanolamine and cholesterol yielded very low exchange activities (~ 0.2 nmol $mg^{-1}sec^{-1}$). In order to obtain optimal exchange rates, it was necessary to include (a) specific anionic phospholipids and (b) cholesterol. Although inclusion of anionic PL were essential, their potency varied as follows: phosphatidylserine (PS) $\geq$ cardiolipin > phosphatidic acid >> phosphatidylglycerol $\geq$ phosphatidylinositol. Maximal exchange activities were found with a combination of PC:PS:cholesterol (30:50:20% by weight). Reconstitution with less PS resulted in lower exchange rates. The $K_M(Ca^{2+})$ was about 10 µM with several lipid compositions. Vesicles formed from the lipids extracted from native cardiac membranes had exchange rates lower than those from PC:PS:cholesterol (30:50:20).

The Na^+/Ca^{2+} exchange of native sarcolemmal vesicles can be stimulated, as shown previously, by proteinase treatment, SDS, high pH, or intravesicular Ca^{2+}. Similar effects could also be demonstrated with reconstituted vesicles. Exceptions were that proteinase and SDS stimulated reconstituted vesicles with sub-optimal exchange activity: that is, vesicles reconstituted without asolectin or with less than 50% PS.

M-Pos367 LOCATION OF 5-IODOACETAMIDOFLUORESCEIN (IAF) IN (Na,K)-ATPase BY FLUORESCENCE ENERGY TRANSFER. R. Aguilar & P.A.G. Fortes, Department of Biology, University of California San Diego, La Jolla, California 92093.

We have measured resonance energy transfer between IAF covalently attached to a -SH group of the (Na,K)-ATPase α-subunit (Kapakos & Steinberg, 1982, BBA 693, 493) and either anthroylouabain (AO) bound to the ouabain site or Trinitrophenyl-ATP (TNP-ATP) bound to the ATP site of the enzyme. The AO → IAF transfer efficiency was 25% and the estimated distance between these probes was 52 Å. Quenching of IAF by TNP-ATP (10 mM EDTA plus either imidazole-HCL pH 7.5 or Pipes-Tris pH 7.0) was saturable and consistent with TNP-ATP binding to a homogeneous site. The K_D values for TNP-ATP quenching of IAF fluorescence were: 43 ± 18 nM (24°C) and 151 ± 13 nM (37°C) with no ligands, 5.2 µM with 100 mM K^+ and 2.9 µM with Mg + Pi + ouabain. ATP (1mM) prevented or reversed IAF quenching by TNP-ATP competitively. The K_D values for ATP were 345 ± 45 nM (24°C) and 1-2 µM (37°C). At saturation the IAF → TNP-ATP transfer efficiencies were 38% and 24% in preparations with 3 and 2.1 µmol AO sites/mg protein, respectively. These values correspond to 86% transfer efficiency because IAF reacts with all α-subunits, but TNP-ATP binds only to functional α-subunits. Measurements of IAF fluorescence decays in the presence and absence of TNP-ATP were consistent with this conclusion. The estimated distance between IAF and TNP-ATP was 20-30 Å. These results indicate that the IAF-reactive SH group is on the intracellular portion of (Na,K)-ATPase, between the ATP and the ouabain sites. (Supported by NIH grant RR-08135)

M-Pos368 CHANGES IN THE Na/K PUMP OF THE LOBSTER CARDIAC GANGLION IN RESPONSE TO IONIZING RADIATION. D. R. Livengood and G. M. Wright. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

The large neurons of the lobster cardiac ganglion were used in an attempt to determine the effect of ionizing radiation on steady state Na/K transport properties. Previous studies from this laboratory have demonstrated the usefulness of the Mullins Noda equations to evaluate some of these properties. A previously published study (Livengood, J. Gen. Physiol. 82: 853-874, 1983) measured control values for Em and pump potential were 59 and 4.7 mV, respectively. The calculated values for $[K]_i$, pNa/pK, and the Na/K coupling ratio (r) were 253 uM, 0.03, and 1.44 (3/2). Freshly dissected ganglia were exposed to a cobalt-60 radiation source at 2000 cGy per minute. Two groups of ganglia were exposed: one at 10,000 cGy and the second at 20,000 cGy. The parameters determined for cells exposed to 10K cGy did not significantly differ from the previously published control values. Seven cells from six ganglia were exposed at 20,000 cGy. The average values for membrane and pump potential were 56 and 1.2 mV. The calculated values for $[K]_i$, pNa/pK, and r were 386 uM, 0.05 and 1.06. The measurements seemed to fall into three categories based on the ouabain-sensitive component of the membrane potential. One cell was essentially normal, three cells showed a reduced pump potential (1.8 to 2.8 mV) and calculated r (1.1 to 1.2), and two cells showed an unexpected membrane hyperpolarization of 3.4 and 4 mV on ouabain addition. The mechanism underlying this membrane hyperpolarization is unclear, but it may reflect either a direct change in the Na/K pump ratio in response to irradiation or a ouabain-sensitive membrane conductance change in the absence of a functioning pump, such as that seen in the frog heart (Fishmeister et al., Pflugers Arch. 406: 340-342, 1986).

M-Pos369 INTERACTION BETWEEN LIGANDS AND PHOSPHOINTERMEDIATES OF NAK-ATPASE. Shizuko Yoda and Atsunobu Yoda, Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin 53706

NaK-ATPase from the electric eel forms three phosphorylated intermediates (EP) with MgATP and Na^+: ADP-sensitive K^+-insensitive EP (E_1P), ADP- and K^+-sensitive EP (E*P), and K^+-sensitive ADP-insensitive EP (E_2P). The EP composition varied with the Na^+ concentration in the fragmental enzyme [Yoda and Yoda, J. Biol. Chem. (1986)]. As one approach to study the ligand effect, we examined the change in the dephosphorylation rate (DR) of each EP by the ligand addition after terminating the EP formation. In the fragmental enzyme, the elevation of Na^+ concentration after terminating the EP formation increased the DR of E_2P, which was estimated in the presence of 0.5 mM ADP. On the other hand, the change of Na^+ concentration did not have an effect on the DR of E_1P, which was measured in the presence of 5 mM KCl. It is known that ouabain reduces the DR of total EP and forms the ADP- and K^+-insensitive (E_1P). When 0.5 mM ADP or 5 mM KCl were present during the dephosphorylation, the changes in the DR of EP by ouabain were not observed. These results support our recent finding [Yoda and Yoda, J. Biol. Chem. in press] that in the various NaK-ATPase proteoliposomes containing 10 mM Na^+ on both sides, only E*P changed to E_1P by digitoxigenin after stopping the EP formation. Although the E_2P has been thought to be the active form for the ouabain binding, it is the E*P in the Na-Mg-ATP system. (Supported by NIH grant HL16549)

M-Pos370 FORMATION OF A Na,K-ATPase•OUABAIN•^{22}Na COMPLEX. Bliss Forbush III, with the technical assistance of Grace Jones and John T. Barberia, Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

We have previously found that ^{42}K or ^{86}Rb ions become "occluded" on Na,K-ATPase upon incubation with ouabain; that is the ions are inaccessible from the medium and are released very slowly from the complex (rate constant ~4 x 10^{-4} s^{-1} at 37°C; Curr. Top. in Membr. and Transport, *19*, 167, 1983). Here we report that ouabain can alternatively trap ^{22}Na in a stable occluded state. In a typical experiment Na,K-ATPase (~5mg/ml) was incubated in 10 µl of 2mM ^{22}Na/125 mM sucrose/15 mM histidine/1 mM EDTA/±1 mM ouabain and various additions for 4 s at 20°C, diluted into 5 ml 25 mM imidazole/100mM K/100mM Na (15°C), filtered and rinsed with the same medium. In the presence of MgATP, more ^{22}Na was bound in the presence of ouabain than in its absence, as measured after 15 s in the 15°C filtration medium. This "stable occluded ^{22}Na" was formed with an apparent Na affinity of ~0.7 mM; its dissociation rate is ~0.01 s^{-1} at 37°C. The amount of ^{22}Na stably occluded was roughly the same as the amount of ^{86}Rb that was occluded in parallel experiments, but we do not yet have confidence in an exact stoichiometry. The rate of formation of the ^{22}Na occluded form is consistent with the rate of ouabain binding. Since ^{22}Na occlusion in the presence of MgATP occured even with 0.1 mM ouabain, in which condition the Na,K-ATPase would require many cycles to be inhibited, it is clear that ouabain does not "catch" the enzyme in the midst of the first cycle, but must bind to the predominant state in the steady state -- in this case presumably E_2-P with ^{22}Na bound at an extracellular high affinity site. Surprisingly however, stable ^{22}Na occlusion is also seen with ^{22}Na, ouabain, and Mg alone, suggesting that ouabain may trap ^{22}Na from the E_1 form. ^{22}Na occlusion was completely blocked by K with an affinity of 0.06 mM ([Na] = 2 mM), indicating competition by occlusion of K. The results are consistent with, but by no means prove, the idea that common sites for occlusion may be occupied either by Na or by K. (Supported by GM-31782).

M-Pos371 Properties of 3-O-Methylfluorescein Phosphate Hydrolysis by the Na,K-ATPase. Richard L. Davis, Dept. of Pharmacology, SUNY Health Science Center, Syracuse, New York 13210. (Intr. by Larry C. Stoner).

3-O-Methylfluorescein phosphate (MFP) is a substrate for the phosphatase activity of canine kidney Na,K ATPase with a K_m much lower than that of other phosphatase substrates, and a ouabain inhibitable K-independent activity which is approximately 20% of the activity at saturating K concentrations. The K_m for K dependent hydrolysis is about 70 μM in the presence of 10mM K and 30 μM in 1 mM K. The K_m for K-independent activity is 9 μM. At 10 mM K, ATP appears to inhibit competitively, with a K_i of around 160 μM, as does TNP-ATP with a K_i of 1.4 μM. Phosphate inhibition in the presence of 10 mM K is also competitive with a K_i of about 1 mM. From a Hill plot the $K_{0.5}$ for K activation is 0.5 mM with an n of 2. Choline and Na both inhibit the K-independent hydrolysis of MFP, and choline also inhibits the K dependent hydrolysis at all concentrations examined out to 100 mM. Na, however, stimulates the K dependent activity out to about 30 mM, beyond which it becomes inhibitory; moreover, the stimulation is present even at saturating K concentrations of 10 mM, although the magnitude of activation is greater at smaller K concentrations. Stimulation by Na is consistent with the (Na+K)-dependent phosphatase mechanism proposed as an alternative pathway to that dependent on the E_2K form of the enzyme. In this mechanism a phosphatase substrate acts in an analogous manner to a nucleotide phosphate and phosphorylates the enzyme. MFP's capacity to interact with the high affinity ATP binding site, its high affinity for the enzyme, and its decreasing K_m value with decreasing K concentration are also consistent with this mechanism. (Supported by NS-05430).

M-Pos372 *EFFECTS OF pH, BUFFERS, AND DIACIDIC COMPOUNDS ON Na,K-ATPase ACTIVITIES.* *Joseph D. Robinson, Dept. of Pharmacology, SUNY Health Science Center, Syracuse, NY 13210.*

Na,K-ATPase preparations (from dog kidney) catalyze also p-nitrophenyl phosphatase reactions in the absence as well as presence of K. Whereas Na,K-ATPase and K-phosphatase activities decrease with declining pH from 7.5 to 5.8, K-independent phosphatase activity increases. These changes are similar with imidazole, histidine, and several Good buffers: they are thus attributable to free H^+, probably by affecting enzyme conformation rather than by H^+ occupying specific cation sites (the $K_{0.5}$ for K is not increased). However, two Good buffers, PIPES and ADA, inhibit K-independent activity strongly, K-phosphatase activity moderately, and Na,K-ATPase activity little; both are diacidic. A family of dicarboxylic and disulfonic acids also inhibit. These inhibitions exceed effects of ionic strength alone, and are not attributable to chelation of Mg. With PIPES (pH 6.5) both the K_i for Na as inhibitor of the K-phosphatase reaction and the $K_{0.5}$ as activator of the Na,K-ATPase reaction are decreased; however, the $K_{0.5}$ for K in the phosphatase reaction is increased whereas that in the Na,K-ATPase reaction is unchanged. Furthermore, with PIPES inhibition by ADP and oligomycin is increased, whereas that by P_i and DMSO is decreased: all consistent with PIPES favoring E_1 enzyme conformations. And with 5-iodoacetamidofluorescein-labeled enzyme the fluorescence responses to Na and K are reduced in PIPES buffer, also consistent with this diacidic compound hindering conformational transitions to enzyme forms required for phosphatase hydrolytic activity. Two conclusions: (a) H^+ activates phosphatase activity in the absence of K by favoring conformations similar to those induced by K, E_2; and (b) diacidic compounds (like PIPES) inhibit by hindering transitions to E_2 conformations. *(Supported by NIH grant NS-05430.)*

M-Pos373 THE INTRINSIC THERMODYNAMIC EFFICIENCY OF THE CARDIAC SARCOLEMMAL Ca^{2+}-Mg^{2+} ATPase IS 100%. Deborah Dixon and Duncan H. Haynes, Department of Pharmacology, University of Miami, Miami, FL 33101.

The thermodynamic efficiency of the bovine cardiac sarcolemmal Ca^{2+}-Mg^{2+}-ATPase was evaluated by comparing the Ca^{2+} gradient established with the ATP/(ADP*Pi) ratio. This evaluation was carried out at external Ca^{2+} (4×10^{-8}M), a value below the Km of 8×10^{-8}M. The Mg-ATP and Pi concentrations were held constant at 0.8 mM and 1 mM, respectively, while ADP concentration was varied to give the desired ATP/(ADP*Pi) ratio. Ca^{2+} uptake was monitored with the fluorescent chelate probe chlorotetracycline. Calibration experiments and determination of the fraction of inside-out vesicles allowed the conversion of fluorescence changes to free internal Ca^{2+} concentrations $[Ca^{2+}]_i$. Active Ca^{2+} uptake is described by the equation $2Ca_o^{2+} (+4Cl_o^-) + ATP \rightarrow 2Ca_i^{2+} (+4Cl_i) + ADP + Pi$. Chloride may be neglected since its concentration ratio is close to 1 under all conditions. Maximal uptake to $[Ca^{2+}]_i$ = 23 mM was measured in absence of ADP, giving infinite ATP/(ADP*Pi). This corresponds to a Ca_i/Ca_o gradient of 5.7×10^5. A gradient one-half as large was obtained at an ATP/(ADP*Pi) ratio of 0.18×10^3 M^{-1}. The square of the Ca^{2+} gradient is shown to be proportional to the ATP/(ADP*Pi) ratio for finite values of the latter. A proportionality constant of 1.6×10^7M is obtained. This compares well with the equilibrium constant for hydrolysis of ATP of 1.1×10^7M under our conditions (0.1 mM Mg^{2+}; 37°C). Thus, the enzyme operates with an intrinsic efficiency of 100%. These results will be discussed in relation to cardiac Ca^{2+} metabolism. Supported by USPHS GM 23900, HL07188, and a grant from the American Heart Association, Florida Affiliate.

M-Pos374 **GLYCOLYSIS CAN FUEL Ca^{2+}-UPTAKE IN A PLASMA MEMBRANE VESICLE (PMV) PREPARATION OF SMOOTH MUSCLE.** **Richard J. Paul, Christopher Hardin, Frank Wuytack, Luc Raeymaekers and Rik Casteels, Depts. of Physiology & Biophysics, University of Cincinnati, OH 45267-0576, USA, and University of Leuven, B-3000, BELGIUM.**

In previous work, we have shown in intact smooth muscles that oxidative and glycolytic metabolism can be independently varied, with J_{O_2} correlated with force and J_{lac} with the Na-pump. More recent evidence has demonstrated that glycolysis and glycogenolysis occur in separate compartments in VSM (Lynch & Paul, Science 222:1344, 1983). We postulated a membrane-bound glycolytic enzyme cascade which fueled membrane ion pumps. We tested this hypothesis by investigating the association of glycolysis and Ca^{2+}-uptake in PMV. Plasma membranes were prepared from pig antrum, using sucrose-density gradient separation with digitonin (Raeymaekers et al., BBA 815:441, 1985). All 8 key glycolytic enzymes investigated were present in PMV ranging in specific activity from 26-1400 nmol/(min·mg PMV protein) including: GAPDH (70), PGK (113) PK (1400), and LDH (44). Further treatment with 600 mM KCl reduces these activities to various degrees (s.a. 3-352), but in all cases appreciable enzymic activity was retained in the PMV. In the presence of Fl,6-diP (1 mM), ADP (0.1), NAD (4), and P_i (0.5) at 37°C, PMV (0.1 mg/ml) will generate NADH to steady state levels of 0.05 to 0.1 mM, and lactate, though variable, at maximum rates between 10-20 nmol/min·mg. This glycolyzing medium will also support Ca^{2+}-uptake by PMV, with accumulations at 30 min being approximately 40% of that supported by 5 mM ATP. The ATP concentration in the glycolyzing media at 30 min is 1-5 μM and the total PMV ATPase is at least 10-fold greater than the glycolytic rate, suggesting that the Ca^{2+} ATPase may be preferentially coupled to glycolytic ATP synthesis. Supported in part by AHA SW Ohio, NIH 23240, NSF Belgian American Program and Training Grant HL 07571.

M-Pos375 INVOLVEMENT OF SULFHYDRYL GROUPS IN KCl-MODULATION OF ATP-DEPENDENT CALCIUM TRANSPORT IN RAT PAROTID BASOLATERAL MEMBRANE VESICLES. Indu S. Ambudkar and Bruce J. Baum, C.I.P.C.B., N.I.D.R., N.I.H., Bethesda, Maryland 20892.

The ATP-dependent Ca^{2+} transport activity in rat parotid basolateral membrane vesicles, previously reported by this laboratory (Biochem.J.227:239,1985), was further charecterized. The addition of 1mM DTT to media during isolation of the membranes increased ATP-dependent Ca^{2+} transport in a mannitol medium(~30%).Addition of 150mM KCl in the assay medium stimulated initial rates of Ca^{2+} transport by ~40% in -DTT membranes and ~80% in +DTT membranes. In the KCl medium +DTT membranes exhibited a 50% higher V_{max} than -DTT membranes, with no change in K_m. The loop diuretic, furosemide, inhibited KCl-stimulated Ca^{2+} transport (K_i=50-100μM) in +DTT membranes.

In the presence of extravesicular K-gluconate(150mM) addition of valinomycin induced ~30% decrease in the initial rate of Ca^{2+} transport in membranes isolated ±DTT, indicating the electrogenic nature of this Ca^{2+} pump. When K-gluconate was replaced with 150mM KCl, valinomycin induced a similar inhibition in +DTT membranes. Under the same conditions, however, ATP-dependent Ca^{2+} transport was stimulated in -DTT membranes by about 30-40%.

The data suggest that a Cl^- dependent K^+ flux, regulated by sulfhydryl groups, maybe associated with the electrogenic ATP-dependent Ca^{2+} transport in rat parotid gland basolateral membranes.

M-Pos376 THROMBIN-INDUCED Ca^{2+} MOBILIZATION AND ITS INHIBITION BY Ca^{2+} ANTAGONISTS. Wenche Jy and Duncan H. Haynes, Department of Pharmacology, University of Miami, Miami, FL 33101.

The thrombin-induced Ca^{2+} mobilization and its sensitivity to various Ca^{2+} antagonists were studied by using Quin2 and chlorotetracycline (CTC) techniques. During thrombin-induced platelet activation Quin2 signal shows a rapid elevation followed by a slow decay of $[Ca^{2+}]_{cyt}$. A parallel measurement of CTC fluorescence shows an initial decrease followed by a slow increase of CTC fluorescence. It is shown that the elevation of $[Ca^{2+}]_{cyt}$ is due to both Ca^{2+} influx and internal Ca^{2+} release. The thrombin-induced Ca^{2+} influx accounts for most of the increased Ca^{2+} concentration in the cytoplasm (over 80% of total increased Ca^{2+} concentration). Various Ca^{2+} antagonists including bepridil, verapamil, Cd^{2+}, and TMB-8 were tested for their potency and selectivity in inhibiting the thrombin-induced Ca^{2+} influx and internal Ca^{2+} release. All the above-mentioned Ca^{2+} antagonists inhibit the thrombin-induced Ca^{2+} influx. The IC_{50} values of bepridil, verapamil, TMB-8, and Cd^{2+} for the thrombin-induced Ca^{2+} influx are 2,19,45, and 50 μM, respectively. On the other hand only bepridil and TMB-8 exhibit blocking effect on the thrombin-induced internal Ca^{2+} release with IC_{50} values of 20 and 50 μM, respectively. The results that TMB-8 inhibits both the thrombin-induced Ca^{2+} influx and internal Ca^{2+} release differ from the generally-accepted view that TMB-8 acts as a selective "internal Ca^{2+} mobilization inhibitor." The inhibitory effect on $[Ca^{2+}]_{cyt}$ by verapamil or TMB-8 is shown to be well correlated with the inhibition of platelet aggregation by these two drugs. The data demonstrate the existence of functional Ca^{2+} channels which contribute most of Ca^{2+} required for platelet aggregation induced by thrombin. Supported by USPHS GM 23900 and a grant from the American Heart Association, Florida Affiliate.

M-Pos377 AN INTRACELLULAR-pH CLAMP FOR QUANTIFYING ACID-BASE TRANSPORT PROCESSES IN INTERNALLY DIALYZED SQUID GIANT AXONS. Walter F. Boron. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

In most animal cells, intracellular pH (pH_i) is regulated by ion transport systems that extrude acid from the cell. The standard way of assessing such transporters is to acutely load the cell with acid and monitor the subsequent recovery of pH_i (i.e., alkalinization) with a microelectrode or dye. The rate of acid extrusion is proportional to the pH_i recovery rate (dpH_i/dt). A weakness inherent in this approach is that it is impossible to monitor the acid-extrusion rate continuously at a fixed pH_i, which is important because the relevant transporters are pH_i dependent. To circumvent this problem, we have devised a pH_i clamp. An axon is internally dialyzed and pH_i is monitored with a microelectrode. The solution perfusing the dialysis capillary is formed by mixing two streams of fluid that are identical expect for their pH: one has a pH of 6.0, the other, 7.6. Both are buffered with 2 mM HEPES and 2 mM MES. The mixing takes place in a low-volume 'hub' to which the dialysis capillary is attached. The flow rates of the acidic fluid (F_{acid}) and the alkaline fluid (F_{base}) are controlled by computer-actuated stepper motors. The computer attempts to drive pH_i to the clamp value (pH 6.8) by adjusting F_{acid} and F_{base}, always keeping $F_{acid} + F_{base} = 5$ µl/min. The clamp drives pH_i from its initial value of ~7.4 to 6.8 in ~6000 sec, after which time pH_i is held within 0.01 of the clamp value and F_{acid} (in HCO_3^- solutions) is about ~3.5 µl/min, and is stable to within about ±1%. In the presence of 12 mM HCO_3^- at pH 8, blockade of acid extrusion by 0.5 mM SITS causes F_{acid} to fall by ~1 µl/min over ~15 min. During this time, pH_i varies from the clamp value by at most ±0.02. Thus, this pH_i clamp is sufficiently rapid, stable and sensitive to permit analysis of acid-base transport in squid axons. (Supported by NIH grant NS18400)

M-Pos378 IONOPHORES, DRUGS, AND EXTERNAL CATIONS INDUCE TRANSIENT CHANGES IN NERNSTIAN DYE DISTRIBUTION WITHIN INDIVIDUAL CELLS. Daniel L. Farkas, Mei-de Wei, and Leslie M. Loew, Department of Physiology, University of Connecticut Health Center, Farmington, CT 06032

Video microfluorometry coupled with digital image analysis was used to monitor the Nernstian distribution of the ethyl and methyl esters of tetramethylrhodamine. These dyes are permeant cations with low membrane partition coefficients, low toxicity and minimal pH sensitivity, which have been recently synthesized in our laboratory. The dyes distribute across the plasma membrane and mitochondrial membrane of cells in proportion to the dictates of the Nernst equation and can therefore be used to monitor variations in the respective potentials simultaneously. For example, HeLa or J774 cells which have been bathed in high potasium solutions containing valinomycin, eventually lose most of their fluorescence as both these membranes depolarize. Interestingly, however, within the first few seconds after exposing the cells to this medium, a dramatic increase in internal fluorescence is obtained. This increase can be inhibited by ouabain suggesting that the electrogenic Na/K pump may be activated under these conditions. Mitochondrial drugs and other ionophores have also been used to treat the cells and both spatial and temporal changes in the dye distribution recorded. (Supported by USPHS grants GM35063 and AI22106).

M-Pos379 TRANSPORT OF AMINO ACIDS IN INTERNALLY DIALYZED BARNACLE MUSCLE FIBERS. Lyle W. Horn, Temple University Health Science Center, Philadelphia, PA 19140

Balanus nubilus fibers were studied at 10°C using internal dialysis to control internal composition. Net efflux measured by direct assay of amino acid concentrations in the external solution agreed with efflux determined simultaneously (same fiber) by means of ^{14}C-amino acids. The sarcoplasmic amino acid composition can be controlled by internal dialysis. Barnacle muscle does not have many amino acid transport systems. Solutes like glycine and α-aminoisobutyrate cross the membrane in either direction at very low, Na-independent rates. There may be a transporter similar to the mammalian System L exchanger. Anionic amino acids such as glutamate (glu) are transported inward at much higher rates than the zwitterions. Glu influx is specific for L-glu, inhibited by cysteic and L-aspartic acids, and insensitive to neural glu receptor agonists. Glu influx is a saturatable function of glu concentration. Na is a cis stimulator and trans inhibitor of influx, affecting the maximal flux but not the apparent affinity. Glu is a trans inhibitor of influx. The system is asymmetric. Glu efflux is approximately a linear function of concentration with a very low apparent permeability. Na trans inhibits, but glu trans stimulates efflux. Results indicate that a fully loaded carrier is the most mobile form for flux, while the empty carrier is the most mobile form for efflux. Glu-free forms of the carrier may be concentrated at the external surface under normal conditions, thereby dedicating the system to an uptake role in vivo. The system may be a useful model for mammalian System X_{AG}. (Supported by NIH Grant NS18868)

M-Pos380 AMINOPHOSPHOLIPID TRANSPORT ACROSS THE PLASMA MEMBRANE OF HUMAN PLATELETS.
David L. Daleke and Wray H. Huestis, Department of Chemistry, Stanford University, Stanford, CA 94305.

Phospholipids of the human erythrocyte and platelet plasma membrane are distributed asymmetrically across the membrane: the choline phospholipids are localized in the membrane outer monolayer, whereas the aminophospholipids are found primarily in the membrane inner monolayer. Utilizing morphological changes induced by aminophospholipids with short, saturated acyl chains, we recently found that, in human erythrocytes, this distribution is maintained by a magnesium and ATP dependent aminophospholipid transporter (*Biochemistry* 24 (1985) 5406).

Platelets undergo morphological changes reminiscent of those seen in erythrocytes upon incubation with sonicated unilamellar vesicles composed of dilauroylphosphatidylcholine (DLPC) or dilauroylphosphatidylserine (DLPS). Within 5 min normal discoid platelets become smaller, more spherical, and extend long filopodia; shapes similar to those induced by platelet activating agents. DLPC treated cells retain this shape for hours, whereas DLPS treated cells revert rapidly (< 10 min) to rounded and indented forms. These shape changes induced by DLPC and DLPS are complementary, dependent on lipid concentration, and are not accompanied by alterations in platelet protein or lipid phosphorylation.

The transbilayer distribution of incorporated lipids was measured using ^{14}C-labeled DLPS and DLPC. Cells incubated with radiolabeled DLPS or DLPC were treated with sonicated dioleoylphosphatidylcholine (DOPC) vesicles. The amount of radiolabel extracted from cells reflects the amount in the plasma membrane outer monolayer. Cells treated with DLPC or those treated with DLPS for short times (5 min) were susceptible to extraction of radiolabel, whereas cells treated with DLPS for longer times (> 10 min) were resistent to radiolabel extraction. This indicates that, whereas DLPC becomes stabily incorporated into the platelet plasma membrane outer monlayer, DLPS rapidly accumulates in the plasma membrane inner monolayer. Human platelets may possess a protein, similar to that in erythrocytes, for transporting aminophospholipids to the cell inner monolayer, thereby maintaining transmembrane phospholipid asymmetry. Supported by USPHS Grant HL 23787.

M-Pos381 CHANGES IN ASTROCYTE ATP CONCENTRATION PRODUCED BY COMPOUNDS WHICH INHIBIT VOLUME CONTROL
James E. Olson, Christyne Lawson, and David Holtzman. Department of Psychiatry and Neurology, Tulane University School of Medicine, New Orleans, LA 70112.

Cerebral Astrocytes from primary culture exhibit a regulatory volume decrease (RVD) following swelling in hypoosmotic medium. We have shown that astrocyte RVD is dependent upon the maintenance of cellular ATP levels (Olson et al., J Cell Physiol 128:209-215, 1986). In this report, we demonstrate a correlation between the effects on astrocyte RVD and on cellular ATP content using compounds which have been shown to be elevated in the serum of Reye's Syndrome patients.

Astrocytes were removed from the culture dish and suspended in either isoosmotic phosphate buffered saline (PBS) or hypoosmotic PBS (containing one-half the concentration of NaCl). Experimental cells were suspended in hypoosmotic medium containing 0.1-3.0 mM octanoate, 10 mM salicylate, or 10 mM NH_4Cl. Control cells were suspended in isoosmotic or hypoosmotic medium with no added compound. NH_4Cl and salicylate, which have little effect on RVD, reduced ATP levels relative to controls by 9.2% and 27.8%, respectively. A 51.6% reduction in ATP concentration was observed with 3 mM octanoate, a concentration which completely inhibits RVD. Octanoate moderately uncoupled electron transport in a dose-dependent manner. Salicylate was a much more effective mitochondrial uncoupler. Thus, while alterations in energy metabolism may contribute to octanoate's inhibition of astrocyte RVD, mitochondrial uncoupling is not an important mechanism of action. These effects may be important in the cytotoxic brain swelling observed in Reye's Syndrome patients.

Supported by grants to J.O. from the National Reye's Syndrome Foundation and NIH (NS-23218).

M-Pos382 PHOSPHATE-SENSITIVE AND MEMBRANE SIDE-SPECIFIC INHIBITION OF THE MITOCHONDRIAL PHOSPHATE TRANSPORT PROTEIN BY 4-AZIDO-2-NITROPHENYL PHOSPHATE.
Cuneyt Bukusoglu and Hartmut Wohlrab, Dept. of Cell Physiology, Boston Biomedical Research Institute, Boston, MA. 02114.

Calf heart mitochondria and submitochondrial particles were labeled with 4-azido-2-nitrophenyl phosphate (ANPP), a photoactive inorganic phosphate analogue (Lauquin et al. (1980) Biochemistry 19, 4620-4626). The phosphate transport protein, the ß subunit of F_1-ATPase, and probably the ADP/ATP carrier, all inorganic and organic phosphate binding proteins, react with ANPP in submitochondrial particles. The phosphate transport protein was purified from the labeled membranes and reconstituted into liposomes. The transport catalyzed by this protein from submitochondrial particles was inhibited maximally by 57% with 110 uM ANPP. The inhibition at 90 uM ANPP (45%) was nine times higher than for the protein purified from labeled intact mitochondria. ANPP at 150 uM did not inhibit phosphate-induced mitochondrial swelling, but inhibited by 36% the reconstituted transport activity. At this concentration it did not label the ß subunit of F_1-ATPase in mitochondria. At 100 uM it blocked completely state 3 (pyruvate-malate) respiration. Inorganic phosphate, succinate, and ADP at 1 mM protected the phosphate transport activity of submitochondrial particles partially from ANPP inhibition. Chloride, sulfate, and acetate had no such protective effect. The results suggest that ANPP differentiates between the cytosolic and matrix side of the phosphate transport protein and that its reactive-site is insufficiently close to the matrix-facing active site to reflect the high specificity towards anions of the phosphate transport protein.

M-Pos383 INTERACTION OF QUININE AND DICYCLOHEXYLCARBODIIMIDE WITH MITOCHONDRIAL K^+ TRANSPORT MECHANISMS. Joyce J. Diwan, Charlest Moore, and Teresa Haley, Biology Department, Rensselaer Polytechnic Institute, Troy, NY 12180-3590.

K^+ is thought to enter and leave mitochondria via separate mechanisms. Quinine partially inhibits unidirectional K^+ flux into respiring rat liver mitochondria, and at high concentrations fully blocks unidirectional K^+ efflux. (Diwan, BBRC 135:830, 1986). Dicyclohexylcarbodiimide (DCCD) increases the apparent K_m for K^+ of the K^+ influx mechanism (Gauthier & Diwan, BBRC 87:1072, 1979). Experiments utilizing ^{42}K and atomic absorption spectroscopy to monitor rates of unidirectional and net K^+ flux show that DCCD also partially inhibits K^+ efflux. For example, pretreatment with 30 nmol DCCD per mg protein decreased the unidirectional K^+ influx rate from 1.10 ± 0.12 to 0.59 ± 0.15 nmol/mg protein(min), while the unidirectional K^+ efflux rate was decreased from 1.42 ± 0.27 to 0.79 ± 0.31. Submitochondrial particles, dissolved in a buffered solution (pH 7.5) including 2% triton X-100 and protease inhibitors, have been applied to columns containing expoxy activated Sepharose 6B (Pharmacia) reacted with quinine. After extensive washing of the quinine affinity columns with a pH 7 medium including 2% triton X-100 plus 100 mM NaCl, elution with triton solutions including 500 mM KCl plus 10 mM quinine, or elution with one of the anionic detergents SDS or cholate, yields predominantly a single protein, estimated via SDS PAGE (Laemmli) to be about 53,000 daltons. Autoradiograms show that pretreatment of the mitochondria with [^{14}C]DCCD, under conditions which result in alteration of K^+ influx and efflux rates, causes labeling of the 53,000 dalton protein. Whether this protein has a role in mediating K^+ influx or efflux remains to be established. (Supported by USPHS Grant GM-20726)

M-Pos384 STRUCTURAL PRINCIPLE OF THREE MITOCHONDRIAL SOLUTE CARRIERS. M.Klingenberg, H.Aquila and Th.Link (Intr. by J.McIntyre)Institute for Physical Biochemistry, University of Munich, 8000 Munich 2, Federal Republic of Germany.

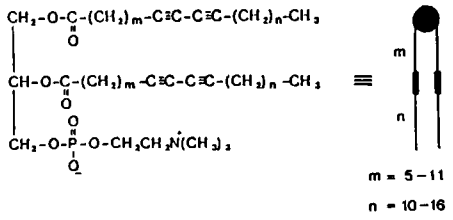
Mechanism of solute transport in mitochondria is understood to an advanced stage on the molecular level. A new insight is achieved by the discovery of homologous triplicate structure of three different mitochondrial carriers. Three mitochondrial solute translocators, ADP/ATP carrier (AAC), the phosphate carrier (PiC) and the uncoupling protein (UCP), i.e. a H^+ carrier, have similar overall structure such as size, hydrophobic behavior, detergent binding, Stokes' radius. The primary structure of these carriers has been first determined by amino acid sequences from mammalian sources and also by cDNA primarily from fungi and plants. The overall homology between these proteins is only between 20 to 30%. All three carriers are constructed according to triplicate structural principle. Each of the carriers has an internal homology between three repeats of about each 100 residues. In the three repeats within each carrier by sequence alignment the conservation of distinct polar aromatic and better turn residues such as glycine and proline is found. The alignment of the nine repeats of all three carriers detects striking conservations even between all repeats. Hydrophobicity analysis also reflects the triplicate principle such that in each segment, a more hydrophobic and a more polar α-segment of around 20 residues are found. In each carrier α-helices probably span six times the membrane. There is probably an additional spanning β-structure. The arrangement of three repeats with each two α-spans around a pseudo three-fold axis as a basic structural principle for these carriers is suggested. Quantitative analysis of mutual homologies in terms of evolution of this carrier family will be discussed.

M-Pos385 CHARACTERIZATION OF POLYMERIZABLE PHOSPHOLIPID TUBULE FORMATION BY CALORIMETRY, FOURIER TRANSFORM INFRARED AND RAMAN SPECTROSCOPIES. Alan S. Rudolph, Thomas G. Burke, and James P. Sheridan, Code 6190, Bio/Molecular Engineering Branch, Naval Research Laboratory, Washington, DC 20375-5000 and Geo-Centers, Inc., Suitland, MD 20746

Large multilamellar vesicles (MLVs) of the polymerizable phospholipid 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-phosphocholine ($DC_{23}PC$) formed by sample hydration above the chain melting temperature (T_M) of 43.3 °C become unstable upon cooling and convert to water-filled multilamellar cylinders referred to as tubules (Yager and Schoen, Mol. Cryst. Liq. Cryst. 106: 371-381, 1984). We have characterized the phase behavior of $DC_{23}PC$ in D_2O and H_2O using differential scanning calorimetry (DSC), FTIR and Raman spectroscopies. Upon cooling at a scan rate of 1 °C min^{-1}, MLVs with outer diameters of 0.5-3 um underwent an exothermic event at 40 °C which corresponded with tubule formation. In contrast, small unilamellar vesicles of $DC_{23}PC$ with diameters of 0.04 um remained metastable in their liquid-crystalline state for several tens of degrees below T_M. At 2 °C, the SUV suspensions converted to a nontubular low temperature phase consisting of large extended sheets of lipid bilayer. DSC heating thermograms of $DC_{23}PC$ showed an endothermic event at 43.3 °C with an enthalpy of 23 kcal mol^{-1}, whereas the corresponding saturated anolog exhibited T_M and enthalpy values of 79.5 °C and 19 kcal mol^{-1}, respectively. FTIR AND Raman results indicated that $DC_{23}PC$ formed a highly ordered, partially dehydrated gel phase. Spectral features of the two different morphologies of the $DC_{23}PC$ low temperature phase were the same. Examination of the C-H stretch region (3000-2800 cm^{-1}) of the $DC_{23}PC$ gel state showed peaks not evident in the spectra of the saturated analog, which may be indicative of the perturbation of methylenes adjacent to the diacetylene. Low frequency Raman data also provided evidence of two distinct methylene populations. These data suggest that tubule formation may be due to special chain packing requirements in the gel state necessitated by the presence of the linear six-carbon diacetylene moiety.

M-Pos386 PHASE BEHAVIOR OF A HOMOLOGOUS SERIES OF POLYMERIZABLE PHOSPHOLIPIDS: EFFECT OF DIACETYLENE GROUP POSITION ON TUBULE FROMATION. Thomas G. Burke, Alan S. Rudolph, Brij P. Singh, James P. Sheridan, Alok Singh, Paul Yager and Paul E. Schoen, Code 6190, Bio/Molecular Engineering Branch, Naval Research Laboratory, Washington, DC 20375-5000 and Geo-Centers, Inc., Suitland, MD 20746

Differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) and Raman spectroscopies, and optical microscopy were used to study the thermotropic phase behavior of aqueous dispersions of seven positional isomers of 1,2-bis heptacosadiynoyl-sn-glycero-3-phosphocholine ($DC_{27}PC$). The homologs differed from one another by the position of the diacetylene group in their twenty-seven carbon long acyl chains. For each lipid studied, large multilamellar vesicles (MLVs) with outer diameters ranging from 0.5-3 um became unstable upon cooling and converted to water-filled multilamellar cylinders referred to as tubules, with DSC cooling scans showing an exothermic event between 49-56 °C associated with the process. Thus, the exact position of the diacetylene group was not critical to tubule formation. DSC heating thermograms of each tubular low temperature phase showed a single transition with chain melting temperatures (T_M) and enthalpy values ranging from 54.9 - 60.7 °C and 26.0 - 29.9 kcal mol^{-1}, respectively. Optical microscopy showed that tubule morphologies were lost at temperatures above T_M. FTIR spectra showed that the CH_2 wagging progression of tubules composed of the various $DC_{27}PC$ homologs exhibited different frequencies as well as intensities. Further characterization of the chain packing of these tubular low temperature phases as well as their polymerization will be reported.



M-Pos387

ORIENTATION OF LIPID TUBULES BY A MAGNETIC FIELD

Charles Rosenblatt[1], Paul Yager[2] and Paul E. Schoen[2]

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Lipid tubules, which are straight hollow cylinders consisting of lipid bilayers, are shown to orient in strong magnetic fields. Birefringence measurements were made of dilute samples of tubules of 1,2-bis(10,12–tricosadiynoyl)-sn-glycero-3-phosphocoline (DC_{23},PC) in magnetic fields of up to 4 T. The tubules were found to orient with their long axes parallel to the field direction, with saturated orientation ($\langle P_2(\cos\theta)\rangle \geq 0.95$) found at about 2 T. From known distributions of lengths and the number of bilayers in the walls, a value $\Delta\chi = (-7 \pm 1) \times 10^{-9}$ erg $cm^{-3}G^{-2}$ was calculated for the tubules, which compares well with some previously reported values for phosphatidylcholines. Alignment of tubules in hydrogels and other polymers locks in the orientation of the tubules. Magnetic alignment will permit more sophisticated structural studies of monomeric and polymeric tubules, and provide a method of orienting macromolecules in the tubule walls or interior.

We gratefully acknowledge partial support by DARPA and the National Science Foundation.

M-Pos388 THE THERMOTROPIC PHASE BEHAVIOUR OF PHOSPHATIDYLCHOLINES CONTAINING dl-METHYL ANTEISO-BRANCHED ACYL CHAINS. DSC, ^{31}P-NMR AND INFRARED SPECTROSCOPIC STUDIES. R.N.A.H. Lewis, B.D. Sykes and R.N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada; C. Madec and H.H. Mantsch, Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada.

The behaviour of phosphatidylcholines (PCs) containing dl-methyl anteisobranched acyl chains was studied by DSC, ^{31}P-NMR and FTIR-spectroscopy. The calorimetric studies show that all of these PCs exhibit a complex behaviour consisting of at least two low-enthalpy, gel-state events which occur at temperatures below that of the gel/liquid-crystalline phase transition. At still lower temperatures, the PCs containing acyl chains with an odd-number of carbon atoms exhibit a 'major' gel-state transition which has been assigned to a conversion from a condensed phase to a loosely packed gel state. No such transition is observed when their even-numbered counterparts are dispersed in water, but in aqueous ethylene glycol, the major gel state transition is discernible. The spectroscopic data indicate that the thermally induced decomposition of the subgel phase involves a major reorganization of the lipid bilayer with distinct changes in the hydrophobic domain, the hydrophobic/hydrophilic interfacial region and the phosphate headgroup of the lipid molecule. The other gel state events were 'seen' by both spectroscopic techniques as a continuous increase in the mobility of the lipid molecules prior to the co-operative melting of the acyl chains. Our data supports the conclusion that the presence of the methyl-anteisobranch results in a relative reduction in the disorder of the liquid-crystalline state and a decrease in the ordering in the gel state. (Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.)

M-Pos389 CALORIMETRIC STUDIES OF A HOMOLOGOUS SERIES OF 1,2-DI-O-ACYL-3-O-(β-D-GLUCOPYRANOSYL)-SN-GLYCEROLS. D.A. Mannock, R.N.A.H. Lewis and R.N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

1,2-di-O-acyl-3-O-(β-D-glucopyranosyl)-sn-glycerols are membrane constituents of several Staphylococci and of Mycoplasma neurolyticum. We have synthesized the entire series of these compounds with acyl chain lengths from 12-20 carbon atoms, for the purpose of studying their thermotropic phase behaviour. Thermograms obtained using a Perkin-Elmer DSC-2C calorimeter at fast heating and cooling rates showed a strongly energetic, lower temperature transition and a weakly energetic, higher temperature transition. These have been assigned to the lamellar phase chain-melting and the lamellar-hexagonal II phase transitions, respectively. The chain-melting transition temperature increases with increasing acyl chain length, whereas the temperature of the Hex_{II} phase transition is relatively constant. On prolonged equilibration at low temperatures, additional gel phases are observed indicating that the gel phase formed on initial cooling is metastable. This transformation is markedly dependent on the acyl chain length and whether they contain an odd- or even-number of carbon atoms. In the shorter chain compounds the transition temperature of the stable gel phase is higher than that of the metastable gel phase, whereas in samples with longer acyl chains the pattern is reversed. Kinetic investigations of samples of the 13:0 and 14:0 compounds have shown the existence of an additional intermediate gel phase, which has a slightly higher transition temperature than the stable form seen on long term storage. The longer chain compounds show no evidence of corresponding behaviour on the time scale of our experiments. Studies of the structure and organization of these stable and metastable gel states with other physical techniques are currently in progress. This should provide insight into the roles of glucosyl diglycerides in biological membranes.

M-Pos390 INTERACTIVE FORCES IN A TERNARY LIPID MIXTURE. J. M. Collins and K. M. Anisur Rahman, Dept. of Physics, Marquette University, Milwaukee, WI 53233 USA.

We have measured the repulsive hydration, attractive van der Waals, and repulsive electrostatic interactive forces in a 1/1/1 molar mixture of dioleoylphosphatidylcholine, dioleoylphosphatidylethanolamine, and Bovine Brain cerebrosides. X-ray diffraction and Synchrotron radiation were used to determine the structural parameters of the arrays, and the osmotic pressure technique of LeNeveu, et. al.(Nature (1976):259) was used in part of the force analysis. As previously observed in other lipid mixtures, the hydration force is significantly altered from that observed for a single species lipid. Also, the attractive force is increased by a greater amount than can be attributed to a van der Waals attraction. Implications of these observations will be discussed.

M-Pos391 A Nuclear Magnetic Resonance Study of the Interaction of Pseudohalides with Liposomes. Gordon L. Jendrasiak, Departments of Radiation Oncology and Physics, East Carolina University and School of Medicine, Greenville, North Carolina, 27858.

The nature of the phospholipid head-group proton NMR signal from liposomes of various compositions has been studied with a 400 MHz NMR instrument. Liposomes composed of both egg phosphatidylcholine and phosphatidic acid exhibit a head-group signal which is a single component. This is in contradistinction to what is found for egg phosphatidylcholine liposomes at both 220 MHz and 400 MHz. Addition of the pseudohalides SCN^- or $SeCN^-$ produced a significant splitting of the head-group signal into two components, in spite of the apparent negative charge on the liposomes. The two components are attributed to head-group CH_3 entities in the liposome interior and on the surface. The addition of the pseudohalide also produced a small but reproducible decrease in the longitudinal relaxation time of the head-group NMR signal. The signal from the P-31 in the phospholipids was unaffected by the anion addition, however, the C-13 peaks from the methyl moieties of the head-group were decreased in intensity upon exposure to the pseudohalides. Since the action of the pseudohalides is thought to be the breaking up of the bound water near the phospholipid head-groups, the splitting may indicate an interaction with CH_3 protons by the anion, as has been proposed for certain organic superconductors.

M-Pos392 ELECTRON DIFFRACTION AND IMAGIMG OF EPITAXIALLY GROWN CRYSTALS OF MONOGALACTOSYLDIACYLGLYCEROL. A. SEN, E.L. HURLEY, S.W. HUI, ROSWELL PARK MEMORIAL INSTITUTE, BUFFALO, NEW YORK 14263; C.M. McConnel and D.L. DORSET, MEDICAL FOUNDATION OF BUFFALO, BUFFALO, NEW YORK 14203.

Epitaxial crystals of sn-3-monogalctosyldiacylglycerol (distearoyl) was grown from solutions in naphthalene and benzoic acid on 400 mesh, carbon coated, electron microscope grids. After crystallization of the lipid, which is directed by the cooled aromatic substrate by lattice matching, naphthalene and benzoic acid were removed by sublimation under high vacuum. The epitaxial crystals were coated with a thin layer of carbon. The grids were examined in JEOL 100B electron microscope. Thin areas were selected for diffraction. Diffraction patterns were recorded on x-ray film. Regions which showed good diffraction patterns were photographed using low dose imaging techniques. The crystals show periodic patterns with repeating electron light and dense lines. The diffraction patterns obtained showed lamellar structures with repeat distance of ~5.4 nm which agrees very well with previous x-ray diffraction studies. Some regions of solution grown crystals were also observed. Those regions show an hexagonal packing for the fatty acids. Patterson function for the epitaxial crystals are determined from the measured intensity of diffraction patterns which have 12 to 15 orders of reflections.

M-Pos393 EFFICACY OF MODEL CHOLESTERYL ESTER RICH VERY LOW DENSITY LIPOPROTEINS (CER-VLDL) AS SUBSTRATES FOR LIPOLYSIS. M.P. Mims and J.D. Morrisett, Baylor College of Medicine, Houston, Tx.

The action of porcine pancreatic phospholipase A_2 on small unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) ± 2% cholesteryl oleate (CO), large CO/DMPC microemulsion particles, and complexes of the vesicles and particles with apoE was measured as a function of temperature. Hydrolysis of DMPC vesicles at their transition temperature, 19°C, was rapid up to ~35% hydrolysis. At 25°C there was an initial lag period followed by a burst of activity. At 37°and 50°C, no more than 6% of the substrate was hydrolyzed during the 10 min. incubation. DMPC vesicles containing 2% CO also showed a transition at 19°C; however, hydrolysis of these particles by PL A_2 was much slower than for DMPC vesicles. At 19°C, hydrolysis of the substrate reached about 5% after 5 minutes and remained constant. Similar results were obtained at 25°, 37°, and 50°C. In addition, DMPC vesicles containing CO were found to be much more stable to fusion than vesicles formed of DMPC alone. When combined with apoE, DMPC vesicles were transformed into smaller particles with a DMPC:apoE ratio of ~11:1 (w:w). At 19°C, hydrolysis of these particles was slow until ~10% of the substrate was digested, when there was a burst of activity. Hydrolysis of the DMPC/apoE complex was rapid at 25° and 37°, reaching 50% or more during the 10 min. incubation. At 50°C, hydrolysis was much slower than at the lower temperatures. Large CO/DMPC microemulsion particles which showed a DMPC surface monolayer transition at 31°C, and a CO core transition at 46°C, were resistant to hydrolysis by PL A_2 at every temperature tested. We conclude that the presence of CO in a DMPC bilayer or monolayer reduces PL A_2 lipolysis by decreasing accessibility of the enzyme to the substrate. (Supported by grants HL-27341, HL-07341 and Welch Q-837).

M-Pos394 THE EFFECT OF FREE CHOLESTEROL ON THE SOLUBILITY OF TRIOLEIN IN PHOSPHOLIPID BILAYERS. Paul J.R. Spooner and Donald M. Small. Biophysics Institute, Housman Medical Research Center, Boston University School of Medicine, Boston, Massachusetts 02118.

Mixtures of egg phosphatidylcholine and cholesterol were co-sonicated with low concentrations of [carbonyl-^{13}C] triolein to form vesicle dispersions in 0.16M KCl at 52°C and then analyzed by ^{13}C-NMR to determine the extent of triglyceride incorporation. The carbonyl resonances for bilayer solubilized triolein were characteristically shifted downfield from those for the excess, non- incorporated material and their equilibrium intensities were compared with those for the phospholipid carbonyl peak to determine the limiting solubility of the triglyceride. The solubility of triolein measured in the phospholipid bilayers (2.1 mol%) was significantly decreased to 1.4 mol%, with respect to phospholipid, at cholesterol levels of just 20 mol% in the bilayer. The triglyceride solubility decreased progressively with increasing cholesterol concentrations to 0.7 mol% at 40 mol% cholesterol and was between 0.1 and 0.2 mol% at the bilayer saturation level of 50 mol% cholesterol. Compared to previous work with cholesteryl oleate, it would appear that triglycerides penetrate cholesterol containing bilayers less readily than the other biologically important class of neutral lipids - cholesterol esters. As with cholesteryl oleate, the major carbonyl peak for bilayer-solubilized triolein shows small upfield shifts with increasing cholesterol, suggesting a somewhat more hydrophobic environment for the triglyceride carbonyls in the presence of free cholesterol. We speculate that the cholesterol content of phospholipid layers may regulate the content of triglycerides and thus their rates of lipolysis in biological systems.

M-Pos395 THE IONIZATION AND DISTRIBUTION BEHAVIOR OF OLEIC ACID IN CHYLOMICRONS AND CHYLOMICRON-SIZED EMULSIONS IN THE PRESENCE AND ABSENCE OF ALBUMIN. Paul J.R. Spooner, Susanne Bennett Clark, Donald L. Gantz, James A. Hamilton, and Donald M. Small. Biophysics Institute, Housman Med. Res. Ctr., Boston University School of Medicine, Boston, Massachusetts 02118.

Rat lymph chylomicrons with a narrow size range, around 100 nm diameter by electron microscopy and column chromatography, were isolated by ultracentrifugation and combined with low levels (0.4 wt%) of [1-^{13}C] oleic acid for NMR studies. The ionization state of the fatty acid and thus its surface (ionized) to core (unionized) distribution within the lipid particles was deduced from the ^{13}C-carboxylic chemical shift. Gross changes in the chemical shift over the pH range 6 to 10 resembled those observed for oleic acid in similarly sized emulsions of triolein and egg phosphatidylcholine and indicated a pH for half-ionization of the fatty acid between 7.5 and 8.0 in both systems, about 3 pH units higher than its expected monomer pKa. Upon introducing bovine serum albumin into these systems, to give 5-6 total fatty acid molecules per albumin, the ^{13}C-carboxylic spectra showed that practically all the oleic acid was associated with the protein at pH 7.4, even when the mass ratio of components was up to 5 in favor of the lipid particles. The results show that partitioning of fatty acids into the cores of large lipoprotein particles can supress markedly the ionization of these species. However, under most physiological circumstances, protein binding should exert a dominant influence on these ionization equilibria and largely determine the clearance of fatty acids from the circulating lipid particles.