T-PM-Pos55 ELECTROSTATIC INTERACTIONS IN THE BINDING SITE OF BACTERIORHODOPSIN. Hillary S Rodman and Barry Honig, Department of Biochemistry, Columbia University, New York, N.Y. 10032

It has recently been suggested that a negatively charged amino acid positioned near the β-ionone ring of the retinal chromophore is responsible for the color of bacteriorhodopsin[1]. The evidence was based on a theoretical analysis of the spectral shifts ("the opsin shifts") observed upon the binding of predesigned retinal analogs (dihydro retinals) to apoprotein. Recently, the opsin shifts of additional synthetic retinals have been reported. These include phenyl, napthyl, 13-trifluoromethyl and a cyanine dye retinal analog. We show that most of the data strongly support the existence of a negative group near the ring while none are in conflict, despite the claims of some workers. The existence of a negatively charged counter-ion to the protonated Schiff base has been a basic assumption of the model. We demonstrate the opsin shifts for the shorter dihydro retinals and the cyanine analog confirms this assumption. The data combined with simple energetic arguments and consideration of the bR sequence strongly suggest that the counter-ion forms a salt-bridge with the protonated nitrogen. Some implications of the electrostatic environment of the binding site are discussed. It is shown that the absorption spectra of the various photocycle intermediates are consistent with a trans-cis isomerization involving motion of the Schiff base end of the chromophore as first suggested by Rosenfeld *et al*[2]. The absorption maxima of protein bound unprotonated Schiff bases such as M_{412} can also be explained in terms of a negative charge near the β-ionone ring. Finally, the red-shifted structured absorption bands observed upon binding various chromophores to the apoprotein can be explained without invoking a ring chain "planarization"[3].

[1]K. Nakanishi *et al*, J.Am.Chem.Soc. 102, 7945 (1982), [2]T. Rosenfeld *et al* J.Appl.Chem. 49, 341 (1977), [3]T. Schrenckenbach *et al* Biochem. 17,5353 (1978). Supported by NSF PCM 82-07145.

T-PM-Pos56 REGULATION OF hR AND sR SYNTHESIS IN *HALOBACTERIUM HALOBIUM* BY RETINAL. *Elena N. Spudich**, *Roberto A. Bogomolni*+, and *John L. Spudich**, *Department of Anatomy, Albert Einstein College of Medicine, Bronx, NY 10461 and +Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

Retinal-deficient (ret⁻) mutants were isolated from $bR^{-}hR^{+}sR^{+}$ and $bR^{-}hR^{-}sR^{+}$ strains of *H. halobium*. Addition of all-*trans* retinal to membranes of such mutants regenerates sR only, as assessed by absorbance measurements, ion translocation measurements, and flash spectroscopy. Examination of the ret⁻mutants grown in the presence and absence of retinal shows that synthesis of the protein component of hR, but not of sR, is controlled by retinal. Addition of exogenous retinal to a retinal-deficient culture triggers synthesis of hR, the kinetics of which can be quantitated by spectroscopic and ion translocation assays. Radiolabeling studies by cyanoborohydride reduction of (^{3}H)retinal in retinal-deficient membranes are in progress, following the method of Lanyi and Oesterhelt (J. Biol. Chem. 257: 2674-2677, 1982). Our preliminary data suggests the chromophoric polypeptide of sR has an apparent molecular weight near 25,000 as determined by SDS-polyacrylamide gel electrophoresis. (Supported by NIH GM 27750 and GM 28767)

bR = bacteriorhodopsin; hR = halorhodopsin; sR = slow-cycling rhodopsin-like pigment.

T-PM-Pos57 THE PURPLE MEMBRANE PROTON PUMP: A MECHANISTIC PROPOSAL FOR THE SOURCE OF THE SECOND PROTON.* Stanley Seltzer and Stanton Ehrenson, Chemistry Department, Brookhaven National Laboratory, Upton, New York 11973.

A mechanistic proposal is presented to account for the observation that two protons are pumped for each turnover in the photocycle of bacteriorhodopsin. Vertical photoexcitation of retinal, its Schiff base, and its protonated Schiff base is known to produce changes in dipole moment such that substantial positive charge develops in the six membered rings of these molecules (Mathies and Stryer, Proc. Natl. Acad. Sci. U.S.A. 73, 2169-73 (1976)). It is suggested that the second proton pumped comes from a Brönsted acid of an amino acid residue which the cyclohexenyl ring of the bound chromophore approaches following vertical excitation. Calculations indicate that the acidity of such a suitably placed Brönsted acid can be increased 3 to 5.5 pK units by virtue of a purely through space electrostatic effect if 0.2 units of additional positive charge develops at C-5 of the chromophore in its excited state. A variant of this mechanism, which will also be discussed, takes account of the sudden polarization effect (Salem, Accts. Chem. Res. 12, 87-92 (1979)) which can generate greater acidity enhancements of the Brönsted acid at comparable or even greater distances from the chromophore. Two other observations: a) the bathochromic shift and b) the deuterium isotope effect in the formation of the first intermediate ($bR_{568} \xrightarrow{h\nu} K_{610}$) are also shown to be consistent with this mechanistic proposal.

* Research supported by the Department of Energy, Office of Basic Energy Sciences.

T-PM-Pos58 EFFECTS OF DEHYDRATION ON THE STRUCTURE OF PURPLE MEMBRANE. James E. Draheim and Joseph Y. Cassim, Dept. of Microbiology, The Ohio State University, Columbus, Ohio 43210.

Dehydration of oriented purple membrane films with dry N_2 or O_2 gas results in the gradual blue shift of the absorption maximum at ca 565 to 540 nm with concomitant decrease in extinction and increase in the band width. Isosbestic points are evident at ca 625 and 525 nm in the dehydration time-course spectra. The circular dichroism associated with this band, attributable to dissymmetric interactions between the retinal and the apoprotein, is also blue shifted from ca 560 to 550 nm with a decrease in ellipticity. However, notably, a shoulder is evident at ca 510 nm in the spectra of the dehydrated films. In concert with these spectral changes are minor changes in the near UV and none in the far UV. Similar blue shifts in the visible spectra of the purple membrane are also apparent in solution when sufficiently high concentrations of polyhydric alcohols are present (80 to 99% by volume). Furthermore, the dehydration effects of films are enhanced when they are impregnated with polyhydric alcohols. Moreover, the far UV circular dichroic spectrum (consisting of a negative band at ca 225 nm and a positive one at ca 196 nm) becomes sensitive to dehydration, resulting in small but significant band shape changes and an approximate 3 nm blue shift of the crossover wavelength. Except for the magnitudes, these changes are identical to the ones previously observed by this laboratory resulting from the bleaching of this membrane. These changes are attributable to a change in the average tilt of the helical axis of the membrane proteins further in-plane and are indicative of the relative metastability of the membrane structure. All the observed hydration effects are reversible. However, the rehydration rate is very much faster than the dehydration one. Possible molecular mechanisms will be discussed.

T-PM-Pos59 EFFECTS OF POLYHYDRIC ALCOHOLS ON THE STRUCTURE OF PURPLE MEMBRANE. James E. Draheim and Joseph Y. Cassim, Dept. of Microbiology, The Ohio State University, Columbus, Ohio 43210.

The oppositely-signed bilobed visible circular dichroic band of the purple membrane, which has been attributed to excitonic coupling of the retinyl transitions, is nonconservative in aqueous solution at neutral pH with the intensity of the positive lobe predominating. Additions of sufficient amounts of polyhydric alcohols result in this band becoming conservative. However, further additions result in the band becoming gradually nonconservative with the negative lobe predominating. Two explanations have been offered to account for this: (1)light-scattering artifacts and (2)conformation change of the apoprotein. Theoretically, excitonic contributions must vanish when the membrane planes are oriented perpendicular to the incident light. In the oriented film spectrum, this band is replaced with a positive Gaussian band. The critical experiment has now been accomplished. When these films are impregnated with polyhydric alcohols, this Gaussian band gradually becomes negative. This change alone can account for the observed changed of the bilobed band. Since the planar retinal is not intrinsically optically active, the Gaussian band must arise from perturbation of the retinal symmetry by the apoprotein or resonance interaction between the retinal and some group on the apoprotein. Therefore, the change in sign of this band must result from a change in conformation of the apoprotein. There is no basis for correlating this change with light-scattering artifacts. Furthermore, there are significant changes in the near UV spectrum in parallel with this change but none in the far UV. Most likely this conformation change involves tertiary structure alterations rather than secondary. In addition, this phenomenon seems to be invariant to the size of the polyhydric acid. However, removal of hydrophilic portions of the apoprotein by enzymatic means enhances it. This suggests that this phenomenon may be a membrane surface effect involving enhancement of hydrophobic interactions.

T-PM-Pos60 RETINAL MIGRATION IS NOT AN ESSENTIAL STEP IN BACTERIORHODOPSIN'S FUNCTION.

A. Fahr§, N.A. Dencher§, N.G. Abdulaev*, A.V. Kiselev*, and A.E. Dergachev* (Intr. by M.P. Heyn). §Dept.of Biophysics, Freie Universität, D-1000 Berlin 33.*Shemyakin Institute, Moscow 11902, USSR.

In order to determine the attachment site(s) of the chromophore retinal in bacteriorhodopsin (BR) and to examine the possible occurrence of light-induced changes in its binding site, we have modified all accessible lysine residues in BR by reductive methylation with formaldehyde and $NaCNBH_3$. Upon transformation of the ε-amino group of the lysyl residues to the ε-N-dimethyl derivatives, these amino acids loose the potential ability to form a Schiff base linkage with retinal. The structure and function of native and modified BR were compared by applying a variety of different biophysical techniques to purple membrane sheets either in aqueous suspensions, or attached to or incorporated into planar lipid bilayers and lipid vesicles, respectively. No differences were observed between native and methylated BR in the kinetics of laser flash induced photocurrents and transmission changes, as well as in the rate and extent of light-induced pH-changes. Furthermore the overall rate, the activation energy, and the extent of light-dark adaptation were not affected by modification. Our results show unambiguously that lysine 216 is the sole attachment site of retinal in BR and that a change in this attachment site does not occur during the photocycle, proton translocation and light-dark adaptation. The ε-amino groups of the lysine residues do not seem to play a crucial rule in the coordinated pathway of proton translocation across bacteriorhodopsin.

T-PM-Pos61 REGENERATION OF NATIVE INTERACTIONS BETWEEN A FRAGMENT OF BACTERIORHODOPSIN AND RETINAL, Erwin London, Mei-June Liao and H. Gobind Khorana. Depts. of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA. 02139

Chymotrypsin cleaves bacteriorhodopsin (BR) into two fragments: C-1 (residues 72-248) and C-2 (residues 1-71). Previous studies have shown that C-1 possesses the retinal attachment site, Lys 216, and approaches retinal at amino acids 193-194, as judged by crosslinking. To further characterize the interaction between C-1 and retinal we have exploited the ability of BR and its fragments to regain native structure and function after complete denaturation. Although renatured C-1 alone will not bind free retinal, a complex of C-1 and retinal which preserves the Schiff's base between retinal and lysine can be isolated from chymotrypsin-treated purple membrane under acidic denaturaing conditions. Refolding of this C-1-retinal complex occurs in dimyristoyl PC/detergent micelles. The refolded complex has a chromophore with λmax=515 nm, different from that of an ordinary protonated Schiff's base of retinal (λmax=445 nm), but different from that of renatured BR as well (λmax= 560 nm). Unlike renatured BR, the C-1-retinal chromophore is rapidly blea ched by light. In addition, we have prepared the C-1-retinyl complex in which the Schiff's base has been reduced by borohydride. After refolding, this species shows intense retinyl fluorescence and fine structure in its absorbance spectrum, properties characteristic of native protein-retinyl interaction. Further studies show maximum refolding occurs at pH 6, the same pH at which refolding of BR is maximal. We conclude that the retinal binding pocket and the main contribution to the interactions responsible for the unique BR chromophore are localized on C-1, but that C-2 influences and stabilizes this interaction as well. Supported by NIH Grants GM28289 and AI11479 and NSF Grant PCM-8110992.

T-PM-Pos62 REGENERATION OF THE NATIVE BACTERIORHODOPSIN STRUCTURE FROM FRAGMENTS. Mei-June Liao, Erwin London, Kuo-Sen Huang and H. Gobind Khorana. Depts. of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

Bacteriorhodopsin (BR) is the first membrane protein that can totally regain native structure and function after complete denaturation. We have used this property to study the folding and reassociation of the fragments of BR. Chymotrypsin cleaves BR to two fragments: C-1, amino acids 72-248 and C-2, amino acids 1-71. Following denaturation in sodium dodecyl sulfate (SDS) both C-1 and C-2 regain secondary structure in phospholipid/cholate/SDS mixed micelles. The secondary structures are very similar to those predicted in native BR. Together, C-1 and C-2 form a complex with a structure resembling that of BR. Retinal binds tightly to C-1 and C-2 complex and thereby stabilizes the folded conformation. The formation of C-1, C-2 and retinal complex occurs maximally at low detergent concentration and has an optimum at pH6. The regenerated chromophore contains two species: one which has absorption properties similar to light-adapted purple membrane and a second which is blue-shifted, with λmax between 450 and 500 nm. The formation of the latter species is favored at higher temperature and is reversible. Vesicles formed from this complex translocate protons at a level close to that of intact BR. BR can also be cleaved by $NaBH_4$ into two other fragments: B-1, amino acids 1-155 and B-2, amino acids 156-248. After seperation and denaturation, these two fragments can reassociate under renaturing conditions to regenerate the native chromophore and restore proton pumping activity. B-1 and C-1 also form a complex with retinal which exhibits the same spectral properties as intact BR. Upon reconstitution into vesicles, the complex of B-1, C-1 and retinal gives high proton translocating activity. This complex seems to have 10 α-helices. Supported by NIH Grants GM28289, AI11479 and NSF Grant PCM-8110992.

T-PM-Pos63 EFFECTS OF CHLORIDE AND pH ON THE CHROMOPHORE AND PHOTOCHEMICAL CYCLING OF HALORHODOPSIN B. Schobert and J.K. Lanyi, Department of Physiology & Biophysics, University of California, Irvine CA 92717.

We have recently demonstrated that the retinal protein, halorhodopsin (HR), in Halobacterium halobium membranes is a light-driven chloride pump (Schobert and Lanyi, 1982, J.Biol.Chem. 257, 10306 -10313). In this study we have examined the effects of chloride and protons on the chromophores of HR and bacteriorhodopsin (BR), with the expectation that these ions, substrates of the two light-driven pumps, respectively, will interact with the apoproteins sufficiently to perturb the retinal moiety. All experiments were with cell envelope vesicles at high salt concentrations which preserved transport activity, i.e. either in 4 M NaCl or in 1.5 M sodium sulfate with added NaCl. We found that in contrast with the photocycle of BR, which showed sharp pH dependence, the photocycle of HR was virtually independent of pH between 5 and 9. Chloride, on the other hand, which did not affect BR under these conditions, had profound effects on the photocycle of HR: the flash yield was greatly enhanced and the overall cycle time was increased about 4-fold. HR, which has an absorption band near 590 nm, exhibited complex pH and chloride dependent spectral shifts not observable in BR. At pH above 8 we found a conversion to a 410 nm species under two conditions: without chloride the shift was reversible and occured in the dark, in the presence of chloride it was poorly reversible and took place only during prolonged illumination. The results can be interpreted in terms of deprotonation of halo-opsin, with subsequent loss of the Schiff's base proton. Thus, chloride appears to raise the pK of the Schiff's base. The affinity constant for chloride in producing these effects is the same as that observed earlier for the light-driven transport.

T-PM-Pos64 SECONDARY STRUCTURE MODELLING OF BACTERIORHODOPSIN, W. Baird, S. Burt, L. Packer and R. MacElroy, Membrane Bioenergetics Group, Department of Physiology-Anatomy, University of California, Berkeley CA 94720 and Extraterrestrial Research, NASA Ames Research Center, Moffett Field CA 94035.

Computer models of possible helix and β-pleated sheet secondary structures for the membrane bound portions of the primary amino acid sequence of bacteriorhodopsin were constructed on the NASA Ames molecular modeling system. Models were analyzed to determine the positions of charged groups and identify the structures which cause these to extend in a common direction. The complete bacteriorhodops monomer was constructed by assigning secondary structures to the seven rods of the three dimensional data according to the several schemes favored by current experimental evidence. For each model, secondary structures were chosen that could be rotationally oriented to exclude the projection of charged side chains into the lipid environment and pair opposing charges internally.

Diffraction amplitudes for two dimensional projections of these models were calculated and compared with the 3.7Å projection found experimentally by Hayward et al., (J. Mol. Biol. 151:491,1982) to see if the theoreticaly preferred orientations gave a better fit than other orientations and whether models with our choice of secondary structures gave a better fit than models composed only of α-II helices.

Further refinement of helix position was carried out using the interactive structural refinement procedure (Zwerling et al., Meth Enzymology 88, 1982) and a best model was selected. Favored models will be presented. Energy minimization techniques will subsequently be used to explore the details of helix packing and side chain positioning.

T-PM-Pos65 ASSIGNMENT OF AMINO ACID SEQUENCE SEGMENTS TO POSITIONS IN THE STRUCTURE OF BACTERIORHODOPSIN
Jill Trewhella, E.P. Gogol, G. Zaccai and D.M. Engelman. Dept. of M.B.&B., Yale University (P.O. Box 6666, New Haven, CT, 06511) and Institut Laue-Langevin, Grenoble, France.

Two dimensional neutron diffraction intensity data have been measured for Bacteriorhodopsin in which specific classes of amino acids are deuterated. Models of Bacteriorhodopsin have been constructed by assigning helical regions in the sequence (1), building them as ideal alpha helices, and aligning the helical segments along axes determined by the rods of density observed in the low resolution electron density map of Henderson and Unwin (2). Models differ from each other in the assignment of helical segments (A-G) to specific rods of density (1-7) and in the rotational orientation of the helices about their axes. To evaluate models a residual factor is calculated between the observed and predicted intensity differences for a given pair of data sets. Calculations have been completed for data set pairs d Val/native; d Val/d Ile; d Phe/native and d Val/d Phe. Only models which place helices F and G in density positions 3 and 4 give good agreement between observed and calculated intensity differences for these data set pairs (see ref. (3) for assignment designation convention). These assignments are inconsistent with previous placements of the sequence in the structure (3,4)

(1) Khorana et al., (1979) Proc. Nat. Acad. Sci. USA., 76:5046-5050
(2) Henderson and Unwin (1975) Nature, 257:28-32
(3) Engelman et al., (1980) Proc. Nat. Acad. Sci. USA., 77:2023-2027
(3) Agard and Stroud, (1981) Biophys. J., 37:589-602

T-PM-Pos66 PH DEPENDENCE OF PROTON PUMPING AND BACTERIORHODOPSIN PHOTOCYCLE INTERMEDIATES IN CELL ENVELOPE VESICLES OF HALOBACTERIUM HALOBIUM.
Li Qing-guo, Rajni Govindjee, and Thomas G. Ebrey, Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The pH dependence of both the light-induced pH changes and the absorption changes due to M412 and 0640 (intermediates of the bacteriorhodopsin photocycle) were measured in bacteriorhodopsin-containing cell envelope vesicles of Halobacterium halobium after single turn-over flashes. ΔpH measurements were made with a combination glass electrode, and the absorption changes were measured with a single-beam kinetic spectrophotometer. The quantum efficiency of proton pumping is highest in the acid range (ph=4-6) and then decreases as the pH is increased. The midpoint of the quantum efficiency change is around pH = 7. As the pH is increased beyond pH =8 the ΔpH increases to a peak at around pH = 9.5. Evidence will be presented which suggests this peak may be due to a high pK (9.5) proton releasing site on the C-terminal side of the purple membrane.

The decay of M412 is biphasic. The amplitude of the slowly decaying component (M^S) was found to be pH dependent with a pK similar to that of the ΔpH. The pH dependence of the fast decaying component (M^F) is opposite to that of M^S and ΔpH. The titration curve for 0640 is similar to that of M^S and ΔpH, in the pH range 5-8. However, the amplitude of 0640 becomes zero around pH 8.

The relationship between proton pumping and the photochemical intermediates is unclear, but 0640 and M^S seem to be likely candidates for coupling between proton pumping and the photocycle. M^S is more likely since a) the quantum yield of both M^S and ΔpH seem to not go to zero at higher pH's and b) the "blue light effect" on proton pumping is attributable to M^S.

T-PM-Pos67 DICYCLOHEXYLCARBODIIMIDE INHIBITS THE PROTON TRANSPORT IN BACTERIORHODOPSIN

Bing K. Jap* and Robert M. Glaeser†, *Donner Laboratory, †Department of Biophysics and Medical Physics, University of California, Berkeley, CA 94720

Dicyclohexylcarbodiimide (DCCD) is well known to inhibit the proton transport by both H^+ ATPase in the membranes of mitochondria, chloroplasts and bacteria, and cytochrome c oxidase. We began the study to see if there is an analogous property of the proton transport in bacteriorhodopsin. DCCD and its water soluble analogues have been reported to react with bacteriorhodopsin and alter its spectroscopic properties. Our preliminary results show that DCCD inhibits proton transport in bacteriorhodopsin reconstituted in phospholipid vesicles under certain preparative conditions. The inhibition of proton transport appears to be accompanied by a change in the absorption spectra of the retinal chromophore. This change occurs even in the dark. It remains to be determined whether the inhibition of the proton transport is light induced, since it is known that bacteriorhodopsin solubilized in Triton X-100 undergoes a light induced reaction with DCCD.

T-PM-Pos68 TRANSVERSE LOCATION OF THE CHROMOPHORE IN PURPLE MEMBRANE VESICLES: DIFFUSION-ENHANCED FLUORESCENCE ENERGY TRANSFER. Richard Leder*, Sam L. Helgerson$^+$ and David D. Thomas. *Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455; $^+$CVRI, University of California Medical School, San Francisco, CA 94131.

We have used diffusion-enhanced fluorescence energy transfer to study the transverse location of the retinal chromophore in the purple membrane from H. halobium. The fluorescent donor in these experiments was Tb^{3+}, chelated by either dipicolinate (net charge -3) or hydroxyethyl-EDTA (net charge 0). The acceptor was the purple membrane protein's retinal chromophore, which absorbs strongly in the wavelength range of terbium emission, resulting in R_o values (distance for 50% transfer efficiency) ranging from 50.3A for the uncharged chelate to 56.8A for the -3 chelate. The membranes of the H. halobium strain used contained no other potential acceptors for Tb. Envelope vesicles were prepared retaining the original membrane asymmetry of the cells. The distance from the chromophore to the internal or external surface was measured by detecting the lifetime of emission from donors in solution inside or outside the vesicles, respectively; and then comparing this lifetime with that observed in a similar experiment using bleached membranes. The results were analyzed as described in previous studies on the retinal chromophore of rhodopsin (Thomas and Stryer, J. Mol. Biol. 154:145-157, 1982).

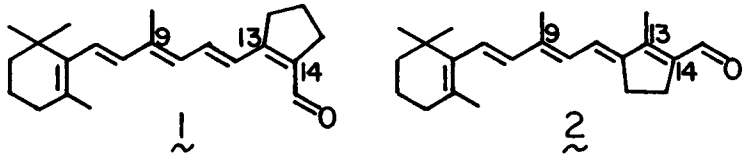
T-PM-Pos69 FTIR DIFFERENCE SPECTROSCOPY OF BACTERIORHODOPSIN AND RHODOPSIN. Kenneth J. Rothschild and Hector Marrero, Departments of Physics and Physiology, Boston University, Boston, MA 02215

We have extended our recent FITR difference measurements of bacteriorhodopsin (K.J. Rothschild and H. Marrero, Proc. Natl. Acad. Sci. USA 79, 4045 (1982)) by examining the effects of isotopic labelling at the Schiff base attachment site of the retinal chromophore. Data is presented on the conformational changes occurring in bacteriorhodopsin during the bR570 to K transition at low temperature as well as the bR570 to M and dark to light-adapted transitions at room temperature. Evidence for the movement of the Schiff base proton away from a counterion and the involvement of a carbonyl group(s) in the primary transition will be discussed. In the case of the photoreceptor membrane isolated from bovine rod outer segments, a similar study has revealed both protein and chromophoric alterations occurring at the bathorhodopsin, Meta I and Meta II stages of bleaching. For example, it is found that one or more water accessible COOH groups are altered during the Meta I to II transition. The general applicability of FTIR difference spectroscopy for detecting conformational changes in other biomembrane systems will be discussed.

T-PM-Pos70 SYNTHETIC ANALOGUES OF BACTERIORHODOPSIN AS PROBES FOR THE MECHANISM OF PROTON PUMPING. Jim-Min Fang, Valeria Balogh-Nair, Koji Nakanishi, Department of Chemistry, Columbia University, New York, N.Y. 10027.

Retinal analogues with fixed 13-cis 1, and fixed 13-trans 2, geometries have been synthesized. Both 1 and 2 formed bacteriorhodopsin analogues with absorption maxima at 548 nm and 576 nm, respectively. Regeneration intermediates displaying fine structure and stable at rt were observed during the reconstitution experiments. When bacteriorhodopsin analogues prepared from 1 and 2 were incorporated into asolectin vesicles and were irradiated continuously at the absorption maxima of the pigments, no alkalinization of the medium was observed. This indicates that the trans to 13-cis isomerization of the retinyl chromophore during the photocycle of bacteriorhodopsin is a prerequisite for proton pumping by purple membrane.

Supported by NSF Grant CHE 81-10505.



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T-PM-Pos71 THERMAL EXPANSION AND ADIABATIC COMPRESSION OF PURPLE MEMBRANE. Jeffrey Marque, C. J. Hardy, Enrico Gratton, Laura Eisenstein, Dept. of Physics, University of Illinois, Urbana, IL 61801.

From measurements of the thermal expansion coefficient $\alpha = 1/V(\partial V/\partial T)_P$, the adiabatic compressibility $\beta = -1/V(\partial V/\partial P)_S$, and the density ρ of concentrated buffered aqueous suspensions of purple membrane from *Halobacterium halobium*, we have calculated the apparent expansibility, compressibility, and density of the purple membrane. Our data, taken together with data from previous studies on globular proteins and lipids, support a simple model in which the protein and lipid molecules expand and compress independently of each other. In addition, our compressibility data suggest that bacteriorhodopsin in native purple membrane binds less water than many globular proteins in neutral aqueous solution, a finding consistent with the lipid surround of bacteriorhodopsin in purple membrane and the preponderance of hydrophobic residues in bacteriorhodopsin.
This work was supported in part by grants from the National Institutes of Health PHS 2 R01 GM18051 and the National Science Foundation PCM 82-09616.

T-PM-Pos72 ELECTROSTATIC BASIS FOR VECTORIAL LIGHT-INDUCED CHARGE SEPARATION IN PHOTOBIOLOGICAL SYSTEMS

Arieh Warshel, Department of Chemistry, University of Southern Calfiornia

The energetics and efficiency of light-induced charge separation across membranes are considered on a molecular level. It is shown how the activation energy that controls the efficiency can be evaluated from the dielectric constant and dielectric relaxation of the system. Examples are given by analyzing the action of artificial and biological photosynthetic systems*, including bacterial reaction center [1,2] and the proton pump of bacteriorhodopsin [3].

1) A. Warshel and D.W. Schlosser, Proc. Natl. Acad. Sci. USA 78, 5564 (1981).
2) A. Warshel, Israel. J. Chem. 21, 341 (1981).
3) A. Warshel, Photochem. Photobiol. 30, 285 (1979).

T-PM-Pos73 PHOSPHOLIPID ASYMMETRY OF THE PURPLE MEMBRANE MEASURED BY UO_2^{2+} QUENCHING OF DANSYL FLUORESCENCE. Robert Renthal and Chung Hwa Cha, U. of Texas at San Antonio, San Antonio, TX 78285

The purple membrane (PM) appears to have a large negative surface charge on the cytoplasmic face, but the actual surface charge density is unknown. We have now devised a method of measuring the relative distribution of phospholipids between the two sides of PM sheets, exploiting the high affinity of $UO_2^{2+}$for phosphate and the effectiveness of UO_2^{2+} in quenching fluorescence. We found the apparent UO_2^{2+} dissociation constant from PM is 0.8 μM for 10 sites per bacteriorhodopsin (BR), and there are 10 moles of phosphate per mole BR. This suggests a 1:1 complex of UO_2^{2+}: phosphate at low concentrations of free uranyl ion. Dansyl hydrazine (DH) was coupled to BR's C-terminal tail (cytoplasmic side; 0.7 moles/mole BR) by a cardodiimide reaction, or to glycolipid (extracellular side; 0.9 moles/mole BR) by periodate oxidation and amino borane reduction. In 2mM acetate buffer, pH 5.6, UO_2^{2+} quenches DH fluorescence with a linear Stern-Volmer plot. By contrast, DH on the cytoplasmic side of PM gives a biphasic quenching curve with a steep slope at low uranyl concentrations. Very little quenching is observed for the DH on the extracellular side. The quenching levels off at a 28% decrease for the cytoplasmic side and a 4% decrease for the extracellular side above 10 μM added UO_2^{2+} (BR = 1 μM). We do not yet know the quenching mechanism at the membrane surface. Without corrections for the differences between the two surfaces in labeling stoichiometry and geometry, the results suggest seven times more phosphate on the cytoplasmic side of the membrane than on the extracellular side. This implies that, at neutral pH, the surface charge density of the cytoplasmic side of PM is greater than -0.01 charges/A^2. (Supported by grants from NSF, NIH, and the Robert A. Welch Foundation.)

T-PM-Pos74 LIGHT-DEPENDENT MODIFICATION OF BACTERIORHODOPSIN WITH TETRANITROMETHANE. Eric Lam†, Stanley Seltzer* and Lester Packer+, Department of Biophysics† and +Membrane Bioenergetics Group, Department of Physiology/Anatomy, University of California, Berkeley, CA 94720; *Department of Chemistry, Brookhaven National Laboratory, Upton, NY, 11973.

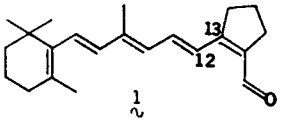
Bacteriorhodopsin (bR) was modified by tetranitromethane (TNM) at pH 8.0 by the method of Lemke and Oesterhelt (Eur. J. Biochem. 115:595-604,1981). This results in a nitrotyrosine peak at about 360 nm and a blue shift in the chromophore. The only nitrotyrosine residue which was not reduced by dithionite was tyr 26 yet the blue shift remained; hence, they concluded that nitration of tyr 26 probably causes the blue shift. We found (Katsura et. al., Biochem. Internatl. 5:445-456) that the blue shift was observed only when light is present during modification. Our results also suggest that modification of another tyr residue requires the presence of light during modification at pH 5.5. Since normal nitration by TNM proceeds with the tyrosinate form of tyr, the fact that low pH did not inhibit this blue shift suggests that the tyr residue modified has an unusual pKa or that a different mechanism of reaction might occur. The fact that this reaction is totally light-dependent at pH 5.5, however, suggests that a complex of TNM with this special tyr might be a factor which causes the observed light-dependency: model system studies with TNM and phenols show that in the presence of light TNM reacts with undissociated phenols (Seltzer, Lam, and Packer, Comm. Ed. JACS, in press). Chymotrypsin cleavage indicated the residue modified was on the C_1 fragment (residues 72-248). This method allows more specific modification of tyr by using low pH and light. Functional characterization will be reported. (Supported by the Divisions of Biological Energy Research and Chemical Sciences, Office of Basic Energy Sciences, Department of Energy.)

T-PM-Pos76 FOURIER-TRANSFORM INFRARED STUDIES OF RHODOPSIN AND ITS PHOTOPRODUCTS AT LOW TEMPERATURE. Kimberly Bagley, Gavin Dollinger, Laura Eisenstein, Joseph Vittitow, Laslo Zimanyi, Dept. of Physics, University of Illinois, Urbana, IL 61801; Thomas G. Ebrey, Burr Nelson, Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

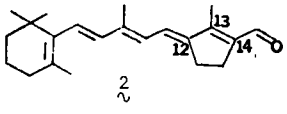
Infrared difference spectra between rhodopsin and isorhodopsin, rhodopsin and bathorhodopsin, and bathorhodopsin and isorhodopsin are presented. A hydrated film of rhodopsin is made by depositing bovine rod outer segment fragments on an IRTRAN 2 (Kodak) window and drying to a film. The film is mounted in a helium refrigerator equipped with NaCl windows. The sample is cooled to 70K and an infrared spectrum is collected at 2 cm^{-1} resolution with a Nicolet 7199 Fourier-Transform Infrared Spectrometer. Photostationary states are obtained by illumination with appropriately filtered continuous light and their infrared spectra collected. The relative concentrations of each species in the photostationary states are deduced by warming the sample to room temperature, resuspending in retinal oxime, and analyzing the isomers present by high performance liquid chromatography. From these relative concentrations infrared difference spectra between any two species can be obtained. Our data for the fingerprint (1400-1100 cm^{-1}), ethylenic (1620-1500 cm^{-1}), and hydrogen out-of-plane (1000-800 cm^{-1}) regions are in agreement with Resonance Raman results. Data in the Schiff base region (1660-1620 cm^{-1}) will be presented and discussed in terms of the state of protonation of rhodopsin and its photoproducts. This work was supported in part by grants NSF PCM 82-09616, HEW PHS GM18051 and EY01323.

T-PM-Pos77 INFRARED AND FLASH PHOTOLYSIS STUDIES OF BACTERIORHODOPSIN ANALOGS CONTAINING RETINALS HAVING FIXED ALL-*TRANS* AND 13-*CIS* GEOMETRIES. Jim-Min Fang, Valeria Balogh-Nair, Koji Nakanishi, Dept. of Chemistry, Columbia University, New York, NY 10027; Chung-ho Chang, Rajni Govindjee, Thomas G. Ebrey, Dept. of Physiology & Biophysics, University of Illinois, Urbana, IL 61801; Kimberly Bagley, Gavin Dollinger, Joseph Vittitow, Laura Eisenstein, Dept. of Physics, University of Illinois, Urbana, IL 61801.

The retinals $\underset{\sim}{1}$ and $\underset{\sim}{2}$ in which the 13-*cis/trans* isomerization is blocked by the 5-membered ring yield the corresponding bacteriorhodopsin analogs, fixed 13-*cis*-bR and fixed all-*trans*-bR.



$\underset{\sim}{1}$



$\underset{\sim}{2}$

Fourier-transform infrared difference studies (70K) of the fixed 13-*cis*-bR analog showed that the photocycle was blocked. Similarly, microsecond flash photolysis studies carried out at toom temperature on fixed all-*trans*-bR indicated no M intermediate formation and no proton pumping. The results of further low-temperature Fourier-transform infrared and flash photolysis studies will be presented. Results so far indicate that the 13-*trans/cis* photoisomerization is essential for both the photocycle and proton pumping. This work was supported in part by grants NSF PCM82-09616, PCM82-01924, CHE 10505, DOE 82 ER 12087, and HEW PHS GM18051.

T-PM-Pos78 Evidence of Asymmetry and Rhodopsin-Phospholipid Interaction in Recombinants. Arlene D. Albert, Department of Biochemistry, 102 Cary Hall, SUNY/Buffalo, Buffalo, NY 14214.

The asymmetry of rhodopsin-phospholipid recombinants formed using pre-formed vesicles was investigated using papain proteolysis and fluorescine-concanavalin A binding. All of the rhodopsin is available to papain proteolysis. This is in contrast to a maximum of only 61% of the rhodopsin in recombinants formed by dialysis techniques (Fong, B.K. and Hubbel, W.L. (1978) Biochemistry 17, 4403). The fragments formed corresponds to those produced by papain cleavage of rhodopsin in the disc membrane. The time course of the reaction however is somewhat slower. Fluorescine-concanavalin A binding studies indicate the carbohydrate moiety is on the interior of the recombinant. Apparent binding to the recombinant is independent of recombinant concentration. After solubilization and reformation of the recombinant (analogous to dialysis techniques) this binding is linearly dependent on recombinant concentration.

The extremely low lipid:protein ratio (40:1) exhibited by these recombinants leads to a perturbed bilayer structure. This is reflected in the extremely short T_1 of 0.14 sec as measured by ^{31}P NMR. The overall shape of the ^{31}P NMR spectrum, however, is typical of bilayer structure and intensity studies have indicated the same number (approximately 15) of phospholipids experienced motional restriction in both the recombinant and in the disc membrane.

T-PM-Pos79 PICOSECOND ABSORPTION AND FLUORESCENCE KINETIC STUDIES OF BOVINE RHODOPSIN WITH A FIXED 11-ENE, J. Buchert*, V. Stefancic, A. G. Doukas, R. R. Alfano, R. H. Callender, J. Pande, Physics Dept., City College of New York, N.Y., N.Y. 10031 and H. Akita, V. Balogh-Nair, K. Nakanishi, Dept. of Chemistry, Columbia University, N. Y., N.Y. 10027

We have incorporated an 11-cis retinal chromophore analog whose 11-12 double bond cannot isomerize with otherwise very similar electronic properties to 11-cis retinal, into the binding site of bovine rhodopsin. Picosecond absorption and fluorescence studies on this non-bleachable rhodopsin have been performed at room temperature for comparison to previous kinetic studies on rhodopsin. The artificial rhodopsin exhibits very different picosecond behavior than rhodopsin. We conclude that isomerization of the retinal moiety is a crucial step in inducing the chain of events in the photolysis of rhodopsin and that this isomerization must occur on the picosecond time scale or faster. This is entirely consistent with the notion that the primary photochemical event in the photolysis of rhodopsin is an 11-cis to trans or transoid photoisomerization.

*On leave from Nonlinear Optics Division, Institute of Physics, A. Mickiewicz University, Poznon, Poland

T-PM-Pos80 ^{13}C SOLID STATE NMR SPECTROSCOPY OF RETINAL AND ITS SCHIFF BASES. Gerard S. Harbison, Judith Herzfeld and Robert G. Griffin, Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115 and Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

Using cross polarization and magic angle sample spinning, we have obtained ^{13}C-NMR spectra, at natural abundance, of all-trans retinal and several of its derivatives. Since at our field (6.9T) and rotation frequencies ($< 3kHz$) we do not fully average the chemical shielding anisotropies, we can measure the principal values of the shift tensors, as well as the isotropic shifts. The isotropic shifts in all-trans retinal correspond closely with those measured in solution, with the following exceptions: (i) the chemically identical prochiral methyl groups C-16 and C-17, which occupy different positions in the crystal, are resolved as a pair of lines 1.5 ppm apart, (ii) the olefinic resonances we assign to C-10, C-11 and C-12 (i.e., the carbons which show the most evidence of strain in the crystal structure) are shifted by 2-5 ppm from their solution positions, and (iii) the methylene resonances of the cyclohexene ring are very disperse, suggesting disorder in the crystal at C-2, C-3 and C-4, in contrast with the x-ray results which were interpreted to indicate disorder at C-2, C-16 and C-17. N-retinylidene butylimine and its protonated derivatives show much more substantial deviations between solid and solution ^{13}C-NMR spectra. Thus, for this group of compounds, theoretical ^{13}C chemical shifts based on x-ray structures can only legitimately be compared with ^{13}C spectra obtained by solid state NMR. The fact that strain and local charge density produce measurable changes in the ^{13}C spectrum, suggests that solid state ^{13}C NMR may help elucidate the environment of retinal covalently linked to protein.

T-PM-Pos81 ^{15}N-NMR OF PROTONATED RETINAL SCHIFF BASES IN THE SOLID STATE. Gerard S. Harbison, Judith Herzfeld and Robert G. Griffin, Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115 and Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

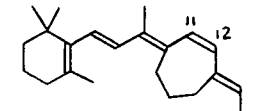
In our recently published ^{15}N-NMR spectra of ϵ-^{15}N-lysyl purple membrane (Biochemistry, in press) we observed a pair of lines at 144 and 151 ppm (downfield from 5.6 M NH_4Cl) deriving from the protonated Schiff base nitrogen. To further develop our interpretation of these spectra, we have measured the shift tensors and isotropic shifts of the ^{15}N-retinylidene butylimmonium salts of phenolic, carboxylic and inorganic acids. We find that, in all cases studied, the variation in the isotropic chemical shift derives essentially exclusively from the variation in σ_{22} and σ_{33}, σ_{11} being confined to the narrow range of 19 to 28 ppm. Significantly, σ_{22} and σ_{33} change together, such that $\sigma_{33} = (157 + \sigma_{22}/1.42)$ ppm, for 12 of the 13 salts. These findings support our view that differences in chemical shifts between Schiff base salts primarily reflect a single variable factor, which we suggest to be the strength of the hydrogen-bond between the Schiff base proton and its counterion. We have also obtained two-dimensional dipolar-chemical shift magic-angle sample spinning spectra of ^{15}N-retinylidene butylimmonium chloride. From these we can derive both the N-H bond lengths and molecular orientations of the ^{15}N chemical shielding tensors. The 1.18 A N-H bond length in the chloride salt is significantly longer than the 1.12 A distance measured for the peptide N-H in glycyl-glycine using identical methods, as well as typical values obtained by neutron-diffraction. The molecular orientations of the chemical shielding tensors correspond closely with those of ^{13}C in electronically isomorphous double bonded systems.

T-PM-Pos82 PROPERTIES OF A cis-CONSTRAINED ANALOG OF THE VISUAL PIGMENT CHROMOPHORE.

D. R. Pepperberg (a), B. R. Nodes (a), J. I. Perlman (a), R. Crouch (b), H. Akita (c), and K. Nakanishi (c). (a) Purdue Univ., W. Lafayette, IN; (b) Medical Univ. of South Carolina, Charleston, SC; (c) Columbia Univ., New York, NY.

In the recently synthesized aldehyde I, the conjugated system of retinal is preserved, but the double bond between carbon atoms 11 and 12 is restricted to a cis configuration by the presence of a 7-membered ring. To investigate the use of I as a probe of the activities of opsin and retinal in situ, we have examined the effects of treatment with I (or the alcohol, II) in bleached ROS and isolated retina preparations of the bullfrog, and in intact eyes of vitamin A-deprived rats.

Spectrophotometry of bleached ROS and isolated retinas (bullfrog): Addition of I promotes the formation of a pigment ($\lambda_{max} \sim 497$ nm) that is slowly degraded by hydroxylamine but relatively resistant to photolysis. In ROS, the analog pigment also forms on the addition of II/$NADP^+$. Vitamin A-deprived rats: I.P. injection of (^{3}H)II induces formation of the analog pigment in the photoreceptors. At 24 h after injection, analog pigment represents ~ 70% of the total amount of opsin (as determined by quantitation of the radiolabel in HPLC-analyzed extracts of opsin chromophores). Formation of ~ 70% analog pigment has no significant effect on threshold of the ERG b-wave. The data suggest that the analog pigment lacks the capacity to promote dark adaptation when formed in functioning photoreceptors. Supported by grants EY-02103 (NIH), EY-00198 (NIH), BNS-80-11563 (NSF), and EY-01253 (NIH).



I R: CHO
II R: CH_2OH

T-PM-Pos83 RHODOPSIN INFLUENCE ON LIPID PHASE BEHAVIOR IN BOVINE ROD OUTER SEGMENT DISK MEMBRANES, Arlene D. Albert, Arindam Sen and Philip L. Yeagle, Department of Biochemistry, 102 Cary Hall, SUNY/Buffalo, Buffalo, NY, 14214.

The phase behavior of bovine rod outer segment disk lipids has been investigated using freeze fracture and ^{31}P NMR. Previously it was reported both that rhodopsin has a possible role in maintaining bilayer structure (deGrip, et al., Biochim. Biophys. Acta 558, 330 (1979)) and that the isolated lipids form bilayers in the presence and absence of rhodopsin (Deese, et al., FEBS Letters 124, 93 (1981)). ^{31}P NMR spectra of isolated disk membranes were taken as a function of temperature between 25°C and 45°C. The characteristic bilayer spectrum was maintained at all temperatures both in the absence of Ca^{+2} and in the presence of 10 mM and 50 mM Ca^{+2}. A similar study was performed on lipids isolated from the disk membranes. In the absence of Ca^{+2} no isotropic phase behavior was observed. In the presence of 10 mM Ca^{+2}, however there was a rapid conversion to the isotropic form. Oxidation of the lipids could not account for this behavior. Removal of the Ca^{+2} at least partially reversed the lipids to the bilayer form. The unsaturation of the disk lipids must be important in this phase response to Ca^{2+}. Mixtures of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine in ratios similar to those found in the disk membrane showed no change from bilayer structure at any of the temperatures or Ca^{2+} concentrations studied.

T-PM-Pos84 IDENTIFICATION OF A PARVALBUMIN-LIKE PROTEIN IN FROG ROD OUTER SEGMENTS. Linda M. Siemankowski and Nancy W. Downer, Department of Biochemistry, University of Arizona, Tucson, AZ. 85721.

An antiserum to frog muscle parvalbumin was used to investigate the presence of parvalbumin-like proteins (cross-reacting) in purified rod outer segment (ROS) membranes and other fractions derived during the purification of ROS membranes. Rabbits were immunized with the 12,000 and 14,000 M_r components of parvalbumin and the resulting antiserum exhibited precipitin lines (Ouchterlony double immunodiffusion) against parvalbumin but not troponin-C or calmodulin. The parvalbumin antiserum precipitated two major proteins (M_r = 12,800 and $M_r \sim$ 16,000) from both crude and purified ROS membrane preparations. No cross-reactivity was observed between the antiserum and a) proteins in concentrated ROS washes or b) low molecular weight, heat stable proteins isolated from sucrose gradients used in the ROS purification. (The latter fraction should include a variety of Ca^{++}-binding proteins present in cells). These results suggest that at least one parvalbumin-like protein co-purifies with ROS membranes and is not likely to be a contaminant derived from retinal neurons or other photoreceptor compartments. The characterization and possible roles for parvalbumin-like proteins in ROS function will be discussed.
(This work was supported by NIH grant EY-03105).

T-PM-Pos85 X-RAY DIFFRACTION STUDIES OF THE ROS DISK MEMBRANE. D. Pascolini & J.K. Blasie, Dept.of Chem. & Biochem./Biophys., Univ. of Pa. and S.M. Gruner, Phys. Dept., Princeton Univ.

Electron density profiles of disk membranes isolated from bovine retinal Rod Outer Segments (ROS) have been determined to ~13Å resolution by analysis of the lamellar X-ray diffraction from reversible swelling experiments on oriented multilayers. A humidity generator capable of accurately regulating the humidity of the gas in the specimen chamber was used to vary the specimen water content. This allowed variation of the unit cell repeat over the range of 144 to 165Å. Data were collected on both film and a 2-dimensional TV detector; both detectors yielded identical patterns consisting of relatively sharp reflections of small mosaic spread. A swelling algorithm was used to determine the best phase choices, taken over pairs of data sets at different periodicities, under the assumption of constancy of the bilayer profile. The diffraction patterns changed dramatically when the unit cell repeat reached 150Å; consequently, the low and high periodicity data sets were independently analyzed. The high periodicity data yielded two statistically equivalent phase choices corresponding to two symmetric, but substantially different, membrane profiles. The low periodicity data yielded two phase choices whose corresponding membrane profiles looked very similar to one another and were characterized by asymmetric electron density distributions. One of the above phase assignment was consistent with both the low and high periodicity data in that it preserved the phases of all strongly diffracted orders and the general features of the profiles. The moderate-to-high resolution of the data combined with the fact that the disk protein in our preparation is predominantly rhodopsin permitted the posing of a model for the disk membrane, in terms of the distribution of the lipid and rhodopsin components, that agreed with the profiles calculated at both high and low periodicity. Supported by EY00673, EY02679 and DE-AC02-76EY03120.

T-PM-Pos86 CHARACTERIZATION OF BOVINE RHODOPSIN mRNA AND cDNA. Wolfgang Baehr and Meredithe L. Applebury, Purdue University, Department of Biological Sciences, Lilly Hall of Life Sciences, West Lafayette, IN 47907.

We have synthesized the dodecameric deoxyribonucleotide GTTCATCATGAT which is a possible coding sequence for Asn(-39)·Met·Met·Ile(-42) in the C-terminal end of bovine rhodopsin. This primer enables reverse transcriptase, in the presence of total retinal mRNA, to predominantly synthesize rhodopsin cDNA. Approximately 100 bases of rhodopsin cDNA were sequenced using dideoxynucleotides as chain terminators (F. Sanger et al., 1977). The nucleotide sequence translates to a chain of 30 amino acids that correspond unambiguously to the known sequence (Pro(-46) to Phe(-76)) of rhodopsin (Ovchinnikov et al., 1982). Rho cDNA synthesized in the presence of primer amounts to 3-5% of that primed with oligo-dT indicating a moderate abundance of rhodopsin mRNA in the retina. Northern blots of retinal poly-A mRNA, sized on a denaturing RNA gel or a sucrose gradient, and hybridized with Rho cDNA, reveal that rhodopsin mRNA has a sedimentation coefficient of ~ 21S. This size is consistent with a chain length of > 2000 bases which is more than double that necessary to code for rhodopsin.

F. Sanger, S. Nicklen, and A. R. Coulsen (1977). Proc. Natl. Acad. Sci. USA 74, 5463-5467.

Yu. A. Ovchinnikov et al. (1982). Bioorganicheskaya Khimia 8, 1011-1014.

T-PM-Pos87 LIGHT STIMULATES THE THIOPHOSPHORYLATION OF THE RIM PROTEIN IN THE DISCS OF ROD PHOTORECEPTORS. E. Z. Szuts, Marine Biological Lab., Woods Hole, Ma. 02543.

The protein kinases of rod photoreceptors resemble other kinases in their ability to thiophosphorylate proteins when their normal substrate donor, ATP, is replaced with ATP-γ-S. The advantage of thiophosphorylation is that thiophosphate groups can not be subsequently cleaved from proteins by phosphatases. Thus, the thio-analogs of normally short-lived phosphorylated products can be identified.

Isolated rod outer segments from frogs were incubated with either [^{35}S]ATP-γ-S or [^{35}S]GTP-γ-S. Radioactively labelled proteins were identified by electrophoresis on SDS-acrylamide gels, followed by fluorography. Light stimulated the thiophosphorylation of two proteins: rhodopsin and a high molecular weight peptide. This latter protein migrates with a molecular weight of 250K on a 4% Laemmli gel system and hence is identified as the rim protein of rod discs (Papermaster et al.,1976 Exp. Eye Res. 23:105; Molday and Molday, 1979, J. Biol. Chem. 254:4653). Only the effect of extensive bleaching has been measured so far. Sucrose and Triton X-100 inhibit the thiophosphorylation reactions.

This study suggests that phosphorylation of the rim protein is a reaction within intact receptors that may have been previously overlooked because of subsequent rapid dephosphorylation. What role its light-stimulated phosphorylation plays in phototransduction, if any, remains to be investigated. (Supported by NIH grant EY02399 and by the Rowland Foundation).

T-PM-Pos88 MODERATE CHANGES OF EXTERNAL CALCIUM AND BUFFER CONCENTRATIONS ALTER THE cGMP CONCENTRATION OF THE TOAD ROD PHOTORECEPTOR CELL WITHIN ITS PHYSIOLOGICAL RANGE. Edward P. Meyertholen, Meegan J. Wilson and Sanford E. Ostroy. Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

The effect of changes in the concentration of calcium and buffer (bicarbonate or HEPES) in the perfusate of the excised toad retina on the electrophysiological properties of the rod photoreceptor and on the cGMP concentrations of the rod outer segment were investigated. In the dark the cGMP concentration of the rod outer segment can be reduced by a factor of 2-3 by calcium concentrations in the range of 0.1 - 5.0 mM and buffer concentrations in the range of 24 - 1 mM. The calcium and buffer effects on the cGMP concentration are interdependent. The decrease in cGMP concentration observed in these experiments is in the proper direction and concentration range as is normally observed on illumination of rod photoreceptors. This technique was used to isolate the effect of calcium and cGMP on the electrophysiological properties of the toad rod photoreceptor. In contrast, most other experiments designed to test the effect of calcium on the properties of rod photoreceptors were done under conditions which also altered the internal cGMP concentration. Moreover, major reductions of external calcium concentration (10^{-9} M, 3 mM EGTA) cause large increases in internal cGMP concentration (by factors of 8-19). Adding the phosphodiesterase inhibitor IBMX (5 mM) increases the cGMP concentration by factors of 5-15. Thus most other experiments designed to test the effect of cGMP on the rod photoreceptor were done under conditions of abnormally high internal cGMP concentration or, in contrast to the effect of light, tested the hypothesis by increasing the cGMP concentration of the cell. (Supported by USPHS Grant EY00413 and GM07211.)

T-PM-Pos89 PROTONS RELEASE CALCIUM FROM BROKEN ROD OUTER SEGMENTS. P. Mueller & E. Pugh, Dept. Biochem. & Biophys. and Psychology, Univ. of P., Philadelphia, PA 19104

Increasing the proton concentration outside or inside isolated rods rapidly blocks the dark current of rods (Pugh et al, Invest. Opth. Vis. Sci., 22:80,82) We suggested that protons liberated by the light-activated hydrolysis of cyclic GMP release Ca^{++} ions from intradisk binding sites. Here we report that protons added to the outside of a suspension of broken rod outer segments (ROS) release Ca^{++} from the intradisk spaces. ROS were isolated in darkness from frog retinas by vortexing and filtering through a stainless wire sieve. ROS were syringed through a 22 gauge needle and incubated for 30 min. with 10^{-5} M ^{45}Ca in isotonic solutions of sucrose-KCl mixtures up to 50 mM KCl at pH 7.3, ^{45}Ca uptake was measured after removal of external Ca^{++} by pressure filtration through Dowex 50 ion exchange columns loaded with Tris. Typical uptake was between 0.5 to 2 moles of Ca^{++} per mole of rhodopsin depending on KCl. Addition of protons after incubation resulted in a rapid loss of Ca^{++}, that was faster than the 10 sec. time resolution of measurement. Lowering of the pH from 7.3 to 6.9 caused the loss of 15% of total Ca^{++}; at pH 5.3 about 50% of total Ca^{++} were lost. The exchange stoichiometry for protons liberated within intact rods would probably be higher because the rod suspensions were contaminated with other retinal cell fragments and soluble compounds acting as buffers, and because protons would be liberated at a high local concentration into the interdisk space. But even at a stoichiometry of 10:1 the 10^4 to 10^5 protons expected to be generated per photon would release 10^3 to 10^4 Ca^{++} ions. Supported by NIH Grants EY-02660 and GM-25256.

T-PM-Pos90 Na, K, Cl, Mg, AND EXCHANGEABLE $^{45}Ca^{++}$ IN ROD OUTER SEGMENTS BY COMBINED X-RAY MICROANALYSIS AND RADIOAUTOGRAPHY.

M.C. Foster, J.S. George, B. Trus, and W.A. Hagins Lab. of Chemical Physics, NIADDK and DCRT, N.I.H., Bethesda,Md. 20205

Accumulation of exchangeable Ca^{++} in frog rod outer segments has been demonstrated by a new method. Suspensions of dark-adapted frog rod outer segments containing $^{45}Ca^{++}$ (A_{Ca} 2 μM) were spread as 4 μm layers between tightly stretched 2 μm polyester films and frozen in liquid N_2. One film was peeled off and the suspension freeze-dried. The *trans* side of the remaining film opposite the particles was coated with radioautographic emulsion, exposed for 2-4 days and processed. After coating both sides with carbon, elemental maps of both sides of a film were made in a scanning electron microscope with an energy-dispersive x-ray spectrometer. By comparing the distributions of diffusible elements on the *cis* sides of the films with the Ag on the *trans* sides, sources of radioactivity could be located and their intensities measured. If the ROS were suspended in 110 mM K isethionate containing millimolar levels of ATP, GTP, and creatine phosphate and 10 μM ruthenium red, the rods accumulated $^{45}Ca^{++}$ at least 20-fold over the suspending medium. If the ruthenium red was omitted, most $^{45}Ca^{++}$ was found in widely spaced small particles that were probably free mitochondria. The method is useful for studying distributions of diffusible elements in isolated cells or cell fragments. See *Microbeam Analysis, 1982*, 139-142. A modification of the method is being used to measure the fixed charge density in stacks of rod disks.

T-PM-Pos91 Ca^{++} REGULATION, DARK CURRENT CONTROL, AND Na^{+} GRADIENT ACROSS THE PLASMA MEMBRANE OF RETINAL RODS.

S. Yoshikami, M.C. Foster, and W.A. Hagins Lab. of Chemical Physics, NIADDK, N.I.H., Bethesda,Md. 20205

When the external Ca^{++} activity (A_{Ca}) is stepped from 10^{-3} to 10^{-6}M, the dark current increases from 20 to 100 *pA* for 2-3 s. and then returns to 20 *pA*. If A_{Ca} is stepped back to 10^{-3}M, the dark current shuts down for 30-50 s. and returns to 20 *pA*. Thus the dark current responds to changes in A_{Ca} with large overshoots. Models in which cytoplasmic A_{Ca} is determined by Na-independent or by Na:Ca exchange-dependent extrusion mechanisms are compared with respect to the effects of changes in external [Na^{+}] and [K^{+}] on Ca^{++} efflux, dark current, and cytoplasmic composition in retinal rods. The Na-dependent model, but not the independent model, can account for the complete suppression of the dark current during an upward Ca^{++} step. Changes in cytoplasmic [Na^{+}] predicted by the Na:Ca exchange model were confirmed by x-ray microanalysis of freeze-dried rat rods. At A_{Ca} 10^{-3}, internal [Na^{+}] was 40$\pm$ 5mM. At A_{Ca} 10^{-6} it increased to 110$\pm$ 10 mM. These values were confirmed by determining the values of Na^{+} and K^{+} in the bathing solution required to invert the sign of the dark current in live retinas. We conclude that Na:Ca exchange is the main regulator of cytoplasmic A_{Ca} in retinal rods.

T-PM-Pos92 PHOTOTRANSDUCTION NOISE IN GREEN ROD PHOTORECEPTORS FROM TOAD RETINA. G. Matthews, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Baylor, Matthews & Yau (1980, J. Physiol., 309, 591-621) found that in red rod photoreceptors, events occurred in darkness that were similar to responses to single photoisomerizations. The activation energy for this noise process was similar to that for thermal isomerization of 11-cis retinal, suggesting that the spontaneous dark events were due to thermal isomerization of individual rhodopsin molecules. If this interpretation is correct, the activation energy for spontaneous dark events in other photoreceptors whose visual pigments are based on 11-cis retinal should be similar to that in red rods. To test this, dark noise was recorded from green rods of toad retina. Green rods are physiologically similar to red rods (Matthews, 1982, Neurosci. Abstr., vol. 8, p. 906), and the chromophore of their visual pigment is 11-cis retinal. However, green rod spectral sensitivity is shifted toward the blue (λ_{max} = 433 nm). The rate of spontaneous quantal events in green rods was determined as a function of temperature, and activation energy was estimated from Arrhenius plots of log event rate against reciprocal of absolute temperature. In 6 experiments, the activation energy for spontaneous events was 20.2 $\pm$ 4.1 kcal/mole (mean $\pm$ S.D.). This is similar to the value of 22 kcal/mole previously obtained from red rods. However, the range of activation energy was large in the present study (16.0 - 25.6 kcal/mole), reflecting the difficulty of detecting spontaneous events in background noise in green rods because the single-photon response was about 1/2 to 1/3 the size observed in red rods. Thus, a small difference in activation energy for the process producing spontaneous events in red and green rods cannot be ruled out. (Supported by the Sloan Foundation and by USPHS grant EY03821.)

T-PM-Pos93 PATCH CLAMP RECORDINGS FROM ROD OUTER SEGMENTS. R.D. Bodoia, J. Connor, P.B. Detwiler. Intr. by D.C. Teller. University of Washington, Dept. of Physiol. & Biophys., Seattle WA 98195.

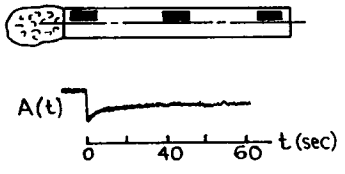
The hyperpolarizing light response of vertebrate photoreceptors depends on cation-selective ionic channels in the cell membrane which open in darkness and close in light. We studied the light sensitive channel using a patch pipet technic (Hamill et al., Pflügers Archiv 391:85-100, 1981). A piece of dark-adapted retina from the nocturnal lizard gekko was perfused with oxygenated Ringers solution and viewed through an inverted microscope equipped with an infrared image converter. The recording pipets, filled with 0.1mM Ca^{++} Ringers, were pressed against the tips of outer segments of intact rods to obtain GΩ seals. Approximately 25% of the patch clamped rods responded to light with an outward change in membrane current. The saturating light response varied from cell to cell over about a tenfold range with a mean value of 1.6pA. The outward photocurrent increased in amplitude with hyperpolarization, decreased with depolarization, and appeared as an inward current at potentials displaced by more than approximately +40mV from the resting potential. The current recorded from a patch of outer segment membrane fluctuated in darkness in an apparently random manner. The peak-to-peak noise was as low as 50fA and contained no discrete events that could be considered the opening or closing of single light sensitive channels. Saturating illumination reduced the current fluctuations by an average value of 1.9 X 10^{-26} A^2. The approximate magnitude of the elementary event underlying the light suppressed noise was estimated by dividing the change in current variance by the change in mean current. The amplitude of the estimated unit event ranged from 4 to 21fA with a mean value of 12fA. Supported by grant EY02048 from the National Eye Institute, USPHS.

T-PM-Pos94 PHOSPHORYLATION AT SITES NEAR RHODOPSIN'S CARBOXYL-TERMINUS REGULATES LIGHT INITIATED CYCLIC-GMP HYDROLYSIS, James L. Miller and Edward A. Dratz, Division of Natural Sciences, The University of California, Santa Cruz, CA 95064.

It has been proposed that phosphorylation at sites near rhodopsin's C-terminus by rhodopsin kinase and ATP quenches the light initiated PDE activation (Liebman, P.A. and Pugh, E.N. [1980] Nature, 287, 734-736). We have made use of a procedure to remove and return several light initiated enzymatic activities including PDE activation and ATP mediated quenching of PDE activation to the ROS. ROS membranes were stripped of extrinsic proteins, portions or all of rhodopsin's phosphorylation sites were removed by proteolysis with trypsin and/or carboxypeptidase Y, and the extrinsic proteins carefully reassociated with the membranes. Rhodopsin which has had most of its C-terminal phosphorylation sites removed retains its ability to activate the cascade of cGMP hydrolysis, but this activation cannot be quenched by ATP. Rhodopsin which has had some of its phosphorylation sites removed is capable of ATP dependent quenching of PDE activation, but this quenching is slower than observed in unproteolyzed membranes. The incorporation of ^{32}P from $\gamma^{32}P$-ATP into disc membranes was greatly reduced in a graded manner by increasing C-terminal proteolysis of rhodopsin. Furthermore, the time required to fully quench PDE activation was linearly related to the incorporation of ^{32}P when the number of sites available for phosphorylation was varied by proteolysis. These results indicate that rhodopsin's C-terminus is not required for PDE activation and phosphorylation of rhodopsin shuts off PDE activation. There are seven phosphorylation sites in the C-terminal region of rhodopsin. Each of these sites is effective in inhibiting PDE activation and the effects of phosphorylation at multiple sites are approximately additive.

T-PM-Pos95 PIGMENT LATERAL DIFFUSION RATES AS A FUNCTION OF DISK MEMBRANE AXIAL POSITION IN RANA PIPIENS ROD OUTER SEGMENTS. Michael W. Kaplan, Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97210

Previous measurement of rhodopsin lateral diffusion rates in Bufo marinus rod outer segment (ROS) disk membranes showed no differences in pigment mobility at different points along the ROS axis.[1] Because birefringence measurements show that the structure of Rana pipiens "red" ROS varies along the cell axis, especially in the basal half of the outer segment, we decided to do similar diffusion measurements using frogs. Dark adapted isolated ROS were positioned on the stage of a kinetic microspectrophotometer relative to a 2 x 6 μm measuring beam aperture using an infrared-sensitive television camera. Beam position was lateral to the center axis, and either in the basal, middle or distal portion of the ROS (see diagram). The aperture was illuminated by dim 531±2nm light, and the transmitted intensity monitored continuously. A bright white pulse (0.5 sec) of light from a xenon arc lamp was superimposed on the measuring beam using a beam splitter, bleaching at least half of the pigment exposed. Rhodopsin diffusion from the unexposed half of the disks produced partial recovery of the measuring beam absorbance. Measurements made in basal, middle and distal ROS showed no significant differences in diffusion-dependent absorbance recovery rates defined as the initial slope of $(A(t) - A(\infty))/(A(0) - A(\infty))$. Therefore the age-dependent structural differences in frog disk membrane structure revealed by birefringence measurements have no measureable effect upon pigment mobility. Supported by USPHS EY01779.



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T-PM-Pos101 STATISTICAL MECHANICS OF MONOLAYERS OF AMPHIPHILIC MOLECULES

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Amphiphilic molecules comprised of a polar head and hydrocarbon tail associate in aqueous environment to form micelles, vesicles, bilayers or other aggregates, consisting of hydrocarbon domains separated from the water regions by the head groups. Statistical theory for the hydrocarbon "interphase' region of such systems is developed, extending previous work to account for chain bending energies and to allow prediction of thermodynamic as well as structural properties. Expressions for the statistical weights of chain configurations and a configurational partition function are obtained subject to the constraint of approximately constant density within the hydrocarbon core, which is a consequence of strongly attractive isotropic van der Waals forces, balanced by hard-core steric repulsive forces. Positional and orientational probability distributions are obtained and evaluated for planar systems for varying chain length, bending energies, and surface areas per chain. Applying Boltzmann's law to the chain partition function gives the chain entropy as a function of the area per chain and temperature. From this entropy we obtain the configurational free energy, from which the contribution to the lateral pressure $\Pi_c(A,T)$ is obtained by taking its areal derivative. $\Pi(A)$ isotherms of lecithin monolayers at a planar oil/water interface are modelled by adding to Π_c expressions for the lateral pressure which result from head group repulsions and van der Waals attractions of the chains. Using Maxwell's equal area construction, the isotherms may exhibit a phase transition, the temperature dependence of which is discussed.

T-PM-Pos102 MASS-ACTION FORMULATIONS OF ION BINDING TO PHOSPHOLIPID MEMBRANES.

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We recently treated the problem of divalent-cation adsorption by single-component charged phospholipid membranes, where the divalent cations adsorb with ion:phospholipid stoichiometries of both 1:1 and 1:2, monovalent cations adsorb with a stoichiometry of 1:1, and di- and monovalent cations either compete or do not compete for membrane binding sites [Biophys. J. 36 (1981), 623]. In the statistical-mechanical treatment only the final equilibrium state of the system need be considered. The equilibrium densities of adsorbed monovalent, 1:1-bound divalent, and 1:2-bound divalent cations were calculated in terms of the aqueous cation concentrations and the cation single-particle partition functions. It is customary, however, to parameterize this problem in terms of mass-action association constants instead of partition functions. This procedure requires assumption of (a) specific kinetic pathways for adsorption and (b) laws of mass action for the interfacial adsorption reactions. We showed that if 1:1 and 1:2 binding of divalent cations are assumed to occur via independent parallel pathways, then under certain conditions laws of mass action are indeed valid. A more likely pathway, however, is a serial one in which 1:2 binding occurs via reaction of a 1:1-bound complex with a nearest-neighbor unoccupied binding site. We show explicitly by statistical mechanics that this two-dimensional lattice reaction obeys a law of mass action. The mass-action equilibrium constants for the serial pathway are related in a simple way to those of the parallel pathway. Adsorption isotherms expressed in terms of the two schemes are formally equivalent. However, they differ in the dependence of 1:2 binding on the 1:1 association constant, as will be shown.

T-PM-Pos103 STRUCTURAL EFFECTS OF CATION BINDING TO PHOSPHATIDYLSERINE BILAYER MEMBRANES.

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The structural properties of dimyristoyl phosphatidylserine bilayers in the anhydrous and hydrated states have been examined using laser Raman spectroscopy. The effects on these properties induced by the monovalent cation Na^+ and the divalent cations Ca^{2+}, Mg^{2+}, Ba^{2+}, and Sn^{2+} have also been studied. The degree of inter- and intra-molecular ordering of the acyl chains in the presence of the various cations was determined by monitoring the relative changes in frequency and intensity of Raman bands in the longitudinal acoustic mode region, optical skeletal, CH_2-bending and CH-stretching regions. Cation-induced dehydration effects were determined by monitoring the carbonyl stretching region and PO_2 stretching bands. The data clearly indicate that CaDMPS is almost totally dehydrated and the acyl chains are in a highly rigid all-trans configuration while NaDMPS is fully hydrated and its bilayer is considerably more disordered with respect to chain conformation and packing. The other cation-DMPS complexes display intermediate properties with respect to dehydration and conformational ordering.

T-PM-Pos104 BACTERIAL LIPOPOLYSACCHARIDES: STRUCTURE AND METAL BINDING STUDIED BY MULTINUCLEAR NMR. S. Michael Strain, Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 3333, SHM-C128, New Haven, Connecticut 06510.

Lipopolysaccharides (LPS) of gram-negative bacteria serve a crucial role in the barrier function of the bacterial outer membrane by virtue of their specific interaction with divalent cations, (Mg^{2+}, Ca^{2+}). Multinuclear NMR techniques were employed to elucidate the structural and metal binding properties of LPS. These studies were directed towards the complete characterization of the chemical structure of LPS; the identification of the functional groups involved in metal coordination; and the elucidation of the conformational and metal binding properties in model membrane systems. ^{13}C NMR was used to determine the linkages and configurations of the saccharide units and the postions of the 3-OH-myristyl groups of native LPS from rough mutant strains of Escherichia coli and Salmonella typhimurium. Resonance assignments were made by chemical shift correlation with model saccharides, lipid A, and deacylated LPS. A significant finding was that in all of the bacterial strains studied, the LPS contained only two residues of 3-deoxy-manno-octulosonic acid (KDO). The results to be presented will include the position and configuration of saccharide linkages in the KDO and lipid A region. The effects of metal binding were studied by ^{31}P, ^{13}C, and ^{111}Cd NMR using native metals (Mg^{2+}, Ca^{2+}), and metal substitutes (Cd^{2+}, Mn^{2+}, Yb^{3+}, Eu^{3+}). Preliminary analysis of this data indicates that the phosphate groups and KDO carboxyl groups are involved in metal binding, which is consistent with the findings in earlier studies using other techniques. A comparison of metal interaction was made between LPS in aqueous dispersions and in reconstituted phospholipid vesicle systems. (NIH Grant AM 18778).

T-PM-Pos105 THE INFLUENCE OF CHOLESTEROL ON STRUCTURAL ORDER AND ACYL CHAIN MOBILITY IN PHOSPHATIDYL CHOLINE MULTILAMELLAR VESICLES. Howard Kutchai, Laura H. Chandler, and George B. Zavoico. Department of Physiology & Biophysics Program, University of Virginia, Charlottesville, VA 22908.

Phase modulation fluorescence spectroscopy of fluorescent probes in phosphatidylcholine (PC) multilamellar vesicles (MLV) was used to study the influence of cholesterol on the acyl chain region of the MLV. Probes used were diphenylhexatriene and 5 anthroyloxy fatty acids: 2,7, 9, and 12-anthroyloxystearate and 16-anthroyloxypalmitate (AP). We estimated fluorescence lifetime, differential polarized lifetime, and steady-state fluorescence anisotropy. We computed the infinite time anisotropy (r_∞) and the probe rotation rate (R). r_∞ is a measure of the structural order of the probe's environment, while R reflects the local resistance to probe rotation. MLV of dipalmitoyl PC (DPPC), dioleyl PC, 1-palmitoyl, 2-oleyl PC, and egg PC were studied with cholesterol concentrations from 0 to 50 mole%. Increased cholesterol tended to increase r_∞ to a much greater extent than it altered R. This suggests that the major effect of cholesterol is to increase the structural order of the PC acyl chains, rather than to increase the 'microviscosity' of the acyl chain region. The effect of cholesterol to increase structural order diminished toward the center of the bilayer: there was no effect of added cholesterol on r_∞ of 16-AP. Effects of cholesterol on R were relatively slight; in some cases R of probes near the bilayer center were increased with addition of cholesterol. In some respects the responses of DPPC-MLV were qualitatively different from the other PCs. This suggests that cholesterol-DPPC systems may not be the best models of the interaction of cholesterol with phospholipids in natural membranes. (Supported by grant RO1 GM24168 from the National Institute of General Medical Sciences.)

T-PM-Pos106 Cross-polarization (CP) ^{31}P NMR of Phospholipids. P.L. Yeagle and J. Frye, Dept. Biochemistry SUNY/Buffalo, Buffalo, NY, 14214 and Dept. Chemistry, Colorado State Univ., Fort Collins, CO 80523

Cross polarization (CP) ^{31}P NMR spectra have been obtained for dimyristoyl phosphatidylcholine, 1-palmitoyl - 2-oleoyl phosphatidylcholine, sphingomyelin and phosphatidylethanolamine hydrated in excess aqueous buffer. These spectra are compared with the CP ^{31}P NMR spectrum of human erythrocyte glycophorin -1-palmitoyl-2-oleoyl phosphatidylcholine recombinants. Spectra were obtained at 60 MHz at room temperature without magic angle spinning. CP ^{31}P NMR spectra of pure phospholipids show a characteristic "hole" in the asymmetric powder pattern. Comparison with the powder patters obtained from normal fourier transform experiments shows that some orientations cross-polarization is ineffective for the pure phospholipids. T_{P-H} and $^1H - T_{1\rho}$ measurements amplify this observation. CP ^{31}P NMR spectra from glycophorin/phosphatidyl-choline recombinants show a different spectral shape. These results are interpreted in terms of a phospholipid headgroup conformation that agrees with a previously proposed model (P.L. Yeagle, Accts. Chem Res. 11, 321 (1978)). It is suggested that this headgroup conformation is significantly changed by high protein content in the membrane. Such a result agrees well with recent T_1 analysis for the same reconbinant system.

T-PM-Pos107 CRYSTAL STRUCTURE OF 2,3-DIPALMITOYL GLYCEROL. W. A. Pangborn and D. L. Dorset (Intr. by V. Cody) Medical Foundation of Buffalo, Inc., 73 High Street, Buffalo, New York 14203.

The crystal structure of 2,3-dipalmitoyl-D-glycerol (DPG) was undertaken as a model for the hydrophobic moiety of the numerous membrane lipids which are derivatives of diacylglycerol. DPG crystallizes in space group $P2_1$ with a=5.486(8)Å, b=7.523(9)Å, c=43.34(5)Å, β=93.68(8)° and Z=2. Of the 2957 independent reflections having θ<75°, 1087 had $F_o > 2\sigma F_o$ and were considered observed. The structure was solved by extending the acyl chains of the recently reported structure of 2,3-dilauroyl-D-glycerol (DLG).[1] The respective final values of unweighted and weighted residuals for all data were 25.3% and 24.4%. For the 1087 reflections considered observed, the corresponding values were 13.0% and 18.9%. The molecules pack tail-to-tail, forming a bilayer structures. There is a H-bond (2.75Å) between the hydroxyl group oxygen and the carbonyl oxygen of the γ-acyl chain of the adjacent molecule translated into *b*. The hydrocarbon chains are packed in the orthorhombic perpendicular subcell. There are no significant differences between the conformation of this compound and that reported for DLG. This homology demostrates the insensitivity of the glycerol dicarboxylic ester conformation to acyl chain length and the efficacy of using the shorter acyl chain compounds as models for naturally occurring phospholipids.

Research was supported in part by grant no. GM21047 from the National Institute of General Medical Sciences, DHHS.

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T-PM-Pos108 COMPOSITIONAL SEGREGATION OF PHOSPHOLIPIDS AT DEFECT REGIONS. M.Y. Tse and M.A. Singer, Dept. of Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Lipid bilayers composed of a binary mixture of saturated phospholipids display broad permeability maxima in the temperature region of phase separation. We have proposed that this enhanced diffusion occurs through defects which develop at domain boundaries and that one of the lipid components of the mixture might specifically congregate at these defects. To explore this postulate we performed the following type of experiment. Liposomes were formed from different molar ratios of two lipid pairs; DSPC-DMPC and DMPE-DMPC. Na22 permeability was measured over an extensive temperature span (15° to 60°). All of the mixtures displayed a permeability maximum in the temperature vicinity of phase separation. For a given mixture we plotted Na22 movement through the defects which develop at phase separation against the fraction of DMPC in the coexisting solid and liquid phases at that temperature (obtained from the phase diagrams). Bilayers of pure DSPC or DMPE show a low permeability to Na22 even at the phase transition. As DMPC is added to the mixture defect permeability remains low until the molar fraction of DMPC in the coexisting solid and liquid phases reaches 0.1;0.4 (for DSPC-DMPC) and 0.35;0.8 (for DMPE-DMPC). These observations suggest that in these binary mixtures, at low fractions of DMPC, the defects are populated by DSPC or DMPE molecules and hence permeability is low. As the fraction of DMPC in the coexisting phases exceeds the values noted above, DMPC congregates at the defects, thus altering their barrier properties. With further increments in the fraction of DMPC, defect permeability rises as these defects become more heavily populated by this phospholipid and mixtures begin to assume permeability characteristics similar to that of DMPC alone.

T-PM-Pos109 HYSTERESIS IN THE PHASE TRANSITIONS OF PHOSPHATIDYLCHOLINE MULTILAMELLAR VESICLES. KW Clubb and BR Lentz. Dept. of Biochemistry, University of North Carolina, Chapel Hill, NC 27514.

High-sensitivity differential scanning calorimetry has been used to characterize the "pre-melting" and "main" phase transitions of 1,2-dimyristoyl-3-sn-phosphatidylcholine and 1,2-dipalmitoyl-3-sn-phosphatidylcholine multilamellar dispersions. Heating and cooling scans were not equivalent for both the pre- and main transitions. For the pre-transition, the position of the excess heat capacity peak was not greatly affected by scan rate, in both heating and cooling scans. We conclude that factors other than the kinetics of the pre-transition must be responsible for at least some of the superheating and supercooling observed. This contrasts with earlier studies stressing the slow rate of the pre-transition (Lentz, Freire, and Biltonen, *Biochemistry* 17, 4475 [1978]; Cho, Chey, and Young, *Biochim. Biophys Acta* 663, 14 [1981]). Surprisingly, the main phase transition peak displayed scan-rate-dependent pre-melting and pre-freezing, in contrast to the supercooling previously reported (Black and Dixon, *Biochemistry* 20, 6740 [1981]). Experiments performed with highly purified gallium as an internal melting point standard demonstrated that this was not an instrumental artifact. Main-transition pre-melting and pre-freezing were greater for higher scan rates (36°C/h) and disappeared at low scan rates (4-10°C/h). This phenomenon has been interpreted in terms of substantial bilayer packing fluctuations that may be kinetically trapped as the phase transition is approached at rapid scan rates. *Supported by NSF Grant PCM 79-22733. BRL is an Established Investigator of the American Heart Association.*

T-PM-Pos110 MONOLAYER, CALORIMETRIC AND ^{19}F NMR STUDIES OF PHOSPHATIDYLCHOLINES CONTAINING BRANCHED-CHAIN FATTY ACIDS. P.M. Macdonald, R.N. McElhaney and K. Poralla, Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7, and Institut für Biologie II, Universität Tübingen, Tübingen, F.R.G.

Monolayers of phosphatidylcholines (PCs) containing methyl iso- and particularly methyl anteiso-branched fatty acyl groups exhibit lower liquid-condensed to liquid-expanded phase transition temperatures than do PCs containing linear saturated fatty acids of comparable chain length. In addition, although branched-chain and n-saturated PCs occupy nearly comparable molecular areas in the liquid-expanded state, the former occupy significantly larger areas in the liquid-condensed state. In fact, cholesterol and bacterial hopanoids can condense monolayers of PCs containing branched-chain fatty acids even below their phase transition temperatures. Branched-chain PC bilayers also exhibit markedly reduced gel to liquid-crystalline phase transition temperatures as measured by differential scanning calorimetry, as well as reduced transition enthalpy and entropy values in comparison to n-saturated PCs of comparable chain length. Thus the low temperature states of PCs containing methyl iso- and particularly methyl anteiso-branched fatty acids appear to be partially disordered in comparison to n-saturated PCs. ^{19}F NMR studies of liquid-crystalline PC bilayers also reveal that iso- and anteiso-branched PCs exhibit lower average orientational order parameter values than do n-saturated PCs when compared at the same absolute temperature, but the opposite is true when compared at temperatures just above their respective transition temperatures. The presence of a methyl branch also appears to extend the plateau region of the order parameter profile. (Supported by the Medical Research Council of Canada and the Deutsche Forschungsgemeinschaft)

T-PM-Pos111 THERMAL STUDIES OF A SUBGEL PHASE IN PHOSPHATIDYLETHANOLAMINES. D.A. Wilkinson and J.F. Nagle, Departments of Physics and Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213

We showed previously (Biochemistry 20, 187 (1981)) using calorimetry and dilatometry that there exists in addition to the usual chain melting transition (T_m) a second phase transition (T_h) in dilauryl phosphatidylethanolamine ($T_h > T_m$). A similar transition has now been observed in dimyristoyl phosphatidylethanolamine (obtained from Avanti Biochemicals). The transition at T_h has larger maximum values of ΔH and ΔV than the one at T_m (ca. 2.5 to 3 times larger). Properly hydrated DLPE or DMPE which has not been incubated at low temperature shows only the reversible gel to liquid crystalline transition usually observed. However, incubation at 2°C results in the appearance of a transition at T_h upon subsequent heating and the concommittant disappearance of the transition at T_m. As with dipalmitoyl phosphatidylcholine, formation of the subgel phase is extremely slow (days). Unlike DPPC, where the gel phase is stable from 13°C to 41°C, it appears that the gel phase in DLPE and DMPE is metastable at all temperatures.

T-PM-Pos112 THE INVERTED HEXAGONAL (H_{II}) PHASE OF PHOSPHATIDYLETHANOLAMINE IS ANTAGONIZED BY HIGH PRESSURE. Paul Yager and Eddie L. Chang. Optical Probes Branch, Code 6512, Naval Research Laboratory, Washington, D.C., 20375

We report here the first measurements of the pressure sensitivity of the transition of a lipid bilayer to a non-bilayer state. Transient non-bilayer states may play a role in modulating the functions of biological membranes. However, little is known about the physical properties of these states. We have developed a novel, accurate method to measure the lamellar to hexagonal II (L-H_{II}) transition of lipids in a pressure cell by monitoring the increase in transmitted light from a laser beam through pelleted samples of lipid dispersions. We find that the L-H_{II} transition of egg phosphatidylethanolamine (EPE) made from egg lecithin is a linear, increasing function of pressure with a slope of 0.0471 C/bar. This value is approximately twice as high as that found for the gel-liquid crystal transition of dipalmitoyl phosphatidylcholine and may be the largest such value for a lipid phase transition. However, using the Clausius-Clapeyron equation, we calculate that the total molar increase in volume is only 4.1 cm^3/mole. The pressure sensitivity of the L-H_{II} transition of EPE can provide a basis for testing for the role of non-bilayer phases in biological phenomena such as membrane fusion and high pressure nervous syndrome. Data for other lipids will also be presented.

T-PM-Pos113 THE DIELECTRIC CONSTANT OF THE MITOCHONDRIAL MEMBRANE. Roland Benz and Stuart McLaughlin. Dept. of Physiology & Biophysics, HSC, SUNY Stony Brook, N.Y. 11794.

Several observations suggest that the bilayer component of the inner membrane of mitochondria has an effective dielectric constant, $\varepsilon_r = 2.7$, that is higher than the value characteristic of solvent-free and decane-containing bilayers, $\varepsilon_r = 2.0$ (McLaughlin & Dilger, Physiol. Rev. 60, 1980). We now propose a simple model that accounts for the ability of the weak acids FCCP, CCCP, and S13 to both transport protons across phospholipid bilayer membranes and uncouple oxidation from phosphorylation in mitochondria (Benz & McLaughlin, Biophys. J., in press). Four parameters are required to characterize this model: the rate constant for the movement of A^- across the membrane, k_A, the rate constant for the movement of HA across the membrane, the adsorption coefficient of A^- onto the membrane solution interface, and the surface pK. These four parameters were determined from kinetic measurements on decane-containing and chlorodecane-containing planar bilayer membranes ($\varepsilon_r = 2.0$ and 2.7, respectively) using the charge pulse and voltage clamp techniques. We confirmed the adequacy of the model by determining each of these parameters independently utilizing equilibrium dialysis, zeta potential, membrane potential, spectrophotometeric, and conductance measurements. As predicted theoretically, the value of k_A depends on both the applied voltage, V, and the dielectric constant of the membrane, ε_r. If oxidation is coupled to phosphorylation by means of a $\Delta\tilde{\mu}_{H^+}$, and $V \simeq 150$ mV, $\varepsilon_r \simeq 2.7$ for the inner membrane of the mitochondrion, the model predicts that FCCP should exert maximal uncoupling activity at a pH$\simeq$ pK. This prediction agrees with the published experimental results.

T-PM-Pos114 LIPID PHASE SEPARATIONS INDUCED BY THE ASSOCIATION OF CHOLERA TOXIN TO PHOSPHOLIPID VESICLES CONTAINING GANGLIOSIDE GM_1. Ernesto Freire, Dept. of Biochemistry, University of Tennessee Knoxville, Tennessee 37916.

The interactions of Cholera Toxin with phospholipid bilayers containing ganglioside GM_1 have been investigated using high sensitivity differential scanning calorimetry. The studies have been performed using large (~900 Å) unilamellar dipalmitoyl phosphatidylcholine vesicles containing ganglioside GM_1 only on the outer monolayer in order to mimic the asymmetric distribution of ganglioside found in the plasma membrane. The incorporation of ganglioside GM_1 in concentrations of up to 10 mole percent causes a small perturbation in the thermotropic behavior of the DPPC vesicles. In the presence of GM_1 the heat capacity function of DPPC becomes broader and skewed towards the high temperature side. The addition of Ca^{2+} acentuates the skewness and at concentrations higher than 10 mM causes the appearance of a second peak at 42.5°C indicating that Ca^{2+} induces phase separation of GM_1. In the absence of Ca^{2+} the addition of Cholera Toxin causes a broadening in the heat capacity function of DPPC-GM_1. The addition of Ca^{2+} to DPPC-GM_1 samples containing Cholera Toxin also causes the appearance of a second peak at 43.5°C; however, under these conditions the area under the high temperature peak is much larger than that observed in the absence of the toxin, suggesting that Cholera Toxin phase separates with ganglioside GM_1 as well as some neutral phospholipid. Deconvolution analysis of the heat capacity function indicates that approximately 60 phospholipids are sequestered per Cholera Toxin molecule even though each toxin molecule specifically binds to only 5 ganglioside molecules. (Supported by NIH Grant GM-30819).

T-PM-Pos115 ETHANOL INDUCED PHASE TRANSITION HYSTERESIS IN PHOSPHATIDYLCHOLINES, Elizabeth S. Rowe, Department of Biochemistry, University of Kansas Medical School, and Veterans Administration Medical Center, Kansas City, MO 64128

We are systematically investigating the interactions of alcohols with individual membrane components and simple mixtures of components in order to elucidate the role of lipid composition in the mechanism of general anesthesia and intoxication. We have shown that ethanol has a biphasic effect on the gel to liquid crystal phase transition of saturated phosphatidylcholines (PC's). At low concentration the lipid transition temperature is lowered with no alteration in transition cooperativity, indicating preferential interactions of ethanol with the fluid phase lipid. At high ethanol concentrations, a secondary interaction occurs which stabilizes the gel phase lipid, raising the transition temperature and increasing the transition cooperativity. Careful studies of the transition reversibility show that in the absence of ethanol or at low ethanol concentrations, heating and cooling curves coincide within one degree. At high ethanol concentrations, however, heating and cooling curves differ in transition midpoint by as much as four degrees. This discrepancy persists even after allowing 17 hours for equilibration. These findings suggest that the secondary low affinity interaction of ethanol with PC involves specific cooperative interactions of ethanol with the head group region of the bilayer.

T-PM-Pos116 ADSORPTION OF PENTACHLOROPHENOL AND CHANGES OF BOUNDARY POTENTIALS OF LECITHIN BILAYERS AND MONOLAYERS. P. Smejtek, R. Jayaweera, K. Hsu and W. Barstad, Environmental Science and Resources Program, Department of Physics, Portland State University, Portland, Oregon 97207.

Electrostatic potential difference across membrane/water interface originates from a layer of charges and oriented dipoles. We have studied changes of electrostatic potential difference due to pentachlorophenol (PCP) by measuring (1) electrophoretic mobility of nonsonicated lecithin vesicles, (2) lecithin monolayer surface potential changes, and (3) PCP adsorption on sonicated vesicles by equilibrium dialysis. The dependence of electrophoretic mobility on PCP concentration can be understood in terms of adsorption model that takes into account the repulsion between PCP anions in solution and charged membrane surface [1]. Our findings suggest that neutral PCP competitively reduces adsorption of PCP anions. The adsorption of PCP on lecithin monolayers decreases electrostatic potential of hydrocarbon region regardless of pH. Monolayer studies indicate that adsorption mechanism and depth of PCP adsorption are similar to that of tetraphenylborate [2].

The boundary potential results provide insight into the properties of PCP-induced membrane conductivity: (a) membrane conductivity saturation occurs within the PCP concentration range corresponding to saturation of electrophoretic mobility, and (b) the excess boundary potential difference of neutral PCP may be the origin of the unexplained shift of PCP-induced membrane conductivity maximum from pH = pK toward high pH.

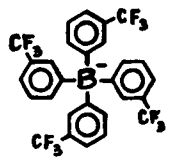
Supported by NIH 5R01 ES00937-08.

[1] S. McLaughlin and H. Harary. Biochemistry. 15:1941:1967.
[2] R.Y. Tsien and S.B. Hladky. Bioph. J. 39:49:1982.

T-PM-Pos117 THERMODYNAMIC ANALYSIS OF HYDROPHOBIC ION BINDING TO PHOSPHATIDYLCHOLINE VESICLES. Ross F. Flewelling and Wayne L. Hubbell, Dept. of Chemistry, Univ. of California, Berkeley, CA 94720

The interaction of hydrophobic ions with membranes is a useful means of investigating electrical aspects of membrane structure and function. Hydrophobic anions like tetraphenylboron (TPB^-) have been extensively studied in planar bilayers. Hydrophobic cations like tetraphenylphosphonium (TPP^+) have received less attention due to their relatively weak binding.

In the present work, we have studied the binding of TPP^+ to sonicated phosphatidylcholine egg (PC) vesicles as a function of temperature and ionic strength. TPP^+ was found to reach electrostatic saturation in the membrane at aqueous concentrations near 600 μM (1 TPP^+/100 lipids). Below saturation its binding constant is determined to be $\beta = 4.1 \pm 0.3 \times 10^{-6}$ cm ($K \simeq 80 \pm 20$). Analysis of the temperature dependence reveals an entropy driven binding $T\Delta S \simeq 6$ kcal/mole at 20°C, with a positive (repulsive) enthalpy of binding, $\Delta H \simeq 3.5$ kcal/mole. If binding saturation is taken to occur where the ion interaction energy is comparable to thermal energy, then TPP^+ and TPB^- data indicates that they bind in a similar dielectric region with $\varepsilon \simeq 10$. TPP^+ binding increases by 25% in going from 0.1 M to 0.5 M NaCl, which may be explained by activity coefficient effects. Although TPB^- and TPP^+ are structural analogs, TPB^- binds 10^3 - 10^4 times more strongly to PC bilayers. The trifluoromethyl-TPB^- analog (I) shown to the right binds nearly as strongly as TPB^- itself. Unlike TPB^-, (I) does not form insoluble salts with choline, at low concentrations, suggesting that the strong binding of TPB^- does not involve specific interactions with the choline moeity of PC.

(I) 

(This work was supported by NIH grants #EY 00729 and T32GM 07379.)

T-PM-Pos118 VESICLE AND NON-VESICLE pH-DEPENDENT BEHAVIOR OF PHOSPHATIDYLETHANOLAMINE DISPERSIONS. C.L. Pryor, L.M.Loew and M. Bridge, Department of Chemistry, State University of New York, Binghamton, New York 13901 USA

Dimyristoylphosphatidylethanolamine (DMPE) and dipalmitoylphosphatidylethanolamine (DPPE) will form vesicles at pH 9. These vesicles can hold entrapped solutes and support a valinomycin K^+ diffusion potential. Fluorescence energy transfer experiments indicate that a phase separation occurs when the pH is dropped to about 6. This is accompanied by precipitation of the lipid. Most striking is that while this precipitation can be reversed by NaOH to return the pH to 9, the phase separation is not reversed. Also, the internal contents of the vesicles are lost during these changes in pH. The pH-dependent precipitation is accompanied by intermixing of the lipid molecules from initially discrete bilayers. This is seen by the non-reversible energy transfer between separately labeled donor and acceptor vesicle populations. Monitoring the fluorescence energy transfer and light scattering during a stopped-flow pH drop shows biphasic kinetics for the energy transfer, but only a single rate for the scattered light change; the slower fluorescence rate corresponds to the observed scattering increase. These results suggest that vesicles formed from DMPE and DPPE are metastable and can exist only at high pH subsequent to ultrasonic irradiation.

(Supported by USPHS Grant GM-25190 and a NIH Research Career Development Award, CA-677 to LML.)

T-PM-Pos119 PERMEABILITY OF ETHER-CONTAINING PHOSPHATIDYLCHOLINE BILAYERS: EFFECT OF CHOLESTEROL. R. Bittman, S. Clejan, L. Fugler, Department of Chemistry, Queens College of CUNY, Flushing, NY 11367, and L. Vargas, A.F. Rosenthal, L.I.J.-Hillside Medical Center, New Hyde Park, NY 11040.

We have reported permeability data with diether-PC bilayers demonstating that the carbonyl oxygens are not needed for PC-cholesterol (Ch) interaction [Biochemistry 18, 2118 (1979); 20, 2790 (1981)]. Others, however, have suggested hydrogen bonding between the Ch β-OH and PC C=O groups. To clarify whether alterations in bonding at the glycerol or phosphocholine moieties of PC exert an influence on interaction with Ch, we synthesized DPPC and DSPC analogs in which an alkyl ether linkage was substituted for one of the ester linkages and phosphinate linkages were substituted for the phosphate. Sonicated vesicles were prepared from: I, 1-O-hexadecyl 2-palmitoyl-PC (an alkyl-acyl-PC); II, 1-palmitoyl 2-O-hexadecyl-PC (an acylalkyl-PC); III, di-C_{16} diether-PC; IV, $ROCH_2CH(OR)CH_2CH_2P(O)(O^-)CH_2CH_2N^+(CH_3)_3$, where R = $C_{18}H_{37}$ (a diether phosphinate PC analog isosteric with the "glycerol" chain); V, $C_{18}H_{37}OCH_2CH(OR)CH_2P(O)(O^-)CH_2CH_2N^+(CH_3)_3$, where R = $C_{16}H_{33}$ (a nonisosteric diether phosphinate). I-III and DPPC formed vesicles of similar sizes. Ch incorporation (25 mol %) into vesicles from I, III, and DPPC decreased the initial rates of valinomycin-mediated $^{86}Rb^+$ efflux by 59-65% and of A23187-mediated $^{45}Ca^{2+}$ efflux by 66-71%; with II, the reduction was about 47%. The first-order rate constant for Rb^+ efflux from vesicles was also decreased by Ch to a greater extent with I, III, and DPPC than with II, showing the nonequivalence of the chains. Ch reduced the initial rate of Rb^+ efflux from vesicles of IV and DSPC to a comparable extent (58-61%); with V the reduction was only 18%, indicating the importance of steric requirements in the glycerol region. Carbonyl oxygens are not required. (Supported by NIH HL 16660)

T-PM-Pos120 RAMAN SPECTROSCOPIC STUDY OF SATURATED MIXED-CHAIN PHOSPHATIDYLCHOLINE MULTILAMELLAR DISPERSIONS. C. Huang,* J. T. Mason† and I. W. Levin, Laboratory of Chemical Physics, NIADDK, National Institutes of Health, Bethesda, Maryland 20205.

The thermotropic behavior of a series of mixed-chain saturated phospholipids in fully hydrated multilamellar dispersions has been examined by vibrational Raman spectroscopy in both the C-C stretching (1000-1200 cm^{-1}) and the C-H stretching (2800-3100 cm^{-1}) mode regions. This lipid series represents PC's in which the sn-1 acyl chain is fixed in length with 18 carbon atoms, while the sn-2 acyl chain length is increased in steps of 2 methylene groups from 10 to 18 carbon atoms. The total changes at T_m in the spectral intensity ratios, $\Delta I(2935\ cm^{-1})/I(2884\ cm^{-1})$ and $\Delta I(1088\ cm^{-1})I(1065\ cm^{-1})$, conform, in general, to a hyperbolic cosine curve with the sequence (18:10)PC> (18:12)PC>(18:14)PC<(18:16)PC<(18:18)PC, with minima in the order/disorder changes for the (18:14)PC species. Structural information from the Raman data lead to the conclusion that the membrane lipids adopt two types of chain conformations in bilayer systems; (a) the two opposing monolayers pack independently, as in the (18:18)PC and (18:16)PC bilayers and (b) an interdigitated arrangement coupling the two monolayers, as in the (18:10)PC, (18:12)PC and (18:14)PC dispersions.

*On sabbatical leave (1981-1982) at NIH. *†Present address: Dept. of Biochemistry, Univ. of Virginia, Charlottesville, Va., 22908.

T-PM-Pos121 SENDAI VIRUS-INDUCED LYSIS OF LIPOSOMES UNDER OSMOTIC STRESS REQUIRES CHOLESTEROL IN ADDITION TO THE VIRUS RECEPTOR. Craig E. Kundrot, Elizabeth A. Spangler, Debra A. Kendall, Robert C. MacDonald and Ruby I. MacDonald. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL. 60201.

Lipid bilayer vesicles were reconstituted with glycophorin, the Sendai virus receptor of human erythrocytes, and loaded with calcein, a polar derivative of fluorescein, at self-quenching concentrations. Upon exposure to Sendai virus and mild hypo-osmotic stress, vesicles of the appropriate composition released a significant portion of their internal contents, as indicated by an increase in calcein fluorescence. Susceptible liposomes were not induced to leak by heat-inactivated virus or by trypsin-treated virus. The response of the vesicles to virus attachment is thus analogous to virus-induced hemolysis and presumably involves fusion of the vesicle and virus membranes. In addition to glycophorin and phosphatidylcholine, cholesterol was absolutely required for the lytic response to the virus. The need for cholesterol was not attributable to inactivation of the virus by liposomes without cholesterol. The presence of gangliosides increased the magnitude of the lytic response, at least by increasing the encapsulated volume of the liposomes, and possibly also by potentiating the receptor activity of glycophorin, but gangliosides did not effectively substitute for glycophorin. Thin layer chromatography of lipid extracted from incubated virus and liposomes, containing a small amount of a fluorescent phosphatidycholine, indicated that phosphatidylcholine in the vesicle is not chemically altered by functional interaction with the virus.

T-PM-Pos122 CONDITIONS OF FREEZING AND THAWING AFFECT INTERVESICULAR MIXING OF MEMBRANE COMPONENTS AS MEASURED BY FLUORESCENCE ENERGY TRANSFER. Ruby I. MacDonald and Robert C. MacDonald. Dept. of Biochem., Mol. Biol. and Cell Biol., Northwestern University, Evanston, IL 60201.

A new combination of fluorescent probes was found suitable for measuring energy transfer--i.e., dansylphosphatidylethanolamine (DaPE) at 2 wt.% and dioctadecylindocarbocyanine (DiI-C_{18}) at 1 wt.% of bulk membrane lipid. In the absence of membrane perturbing conditions--e.g., freezing and thawing--energy transfer between donor (DaPE) and acceptor (DiI-C_{18}) in different sets of vesicles was 5.5 $\pm$ 2.9%. The reversal by EDTA of liposome aggregation induced by Mg^{2+}-dextran sulfate without accompanying change in fluorescence energy transfer showed that energy transfer is not a necessary concomitant of at least some kinds of aggregation. Energy transfer facilitated by freezing and thawing increased with bulk membrane lipid concentration from 2-10 mg/ml, as did light scattering, and with acceptor, but not donor, density in the membrane. The extent of mixing of membrane components originally in different vesicles depended on the conditions under which the vesicles had been frozen and thawed. The conditions either promoting or inhibiting membrane mixing during freezing and thawing were similar to those either promoting or inhibiting the formation of large liposomes brought about by freeze-thawing prior to dialysis (N. Oku and R. C. MacDonald, in press and in preparation). Membrane mixing was enhanced by freezing and thawing in 0.5-1.5 M KCl and 0.5 M KTCA but inhibited by freezing and thawing in 0.1 M KCl, 0.1-1.5 M NaCl, 0.5 M LiCl, 0.5 M glycerol and 0.5 M sucrose. Differences in the mechanisms by which the promoters affect membrane mixing were indicated by the effectiveness of KTCA on both sonicated and unsonicated vesicles, in contrast to the effectiveness of KCl on sonicated liposomes only. (Supported by NIH grant GM 28404).

T-PM-Pos123 Ca^{2+} INDUCED PHASE SEPARATION IN PHOSPHOLIPID MIXTURES. L.J. Lis, J.M. Collins, H. Adamska*, and S.K. Blessing*, Department of Physics, IIT, Chicago, Illinois 60616.

Ca^{2+} solutions of low molarity (7 to 100mM) have been shown by x-ray diffraction to produce phase separations in egg yolk phosphatidylcholine (EYPC) bilayer arrays (Lis, et al, Biochemistry (1981) 20: 1771-1777). One phase was clearly swollen with very large bilayer separations characteristic of charged lipid systems, while the second phase swells to the same limited extend as EYPC in pure water. We have recently examined the effect of Ca^{2+} solutions on egg yolk phosphatidylethenolamine (EYPE) bilayer arrays using x-ray diffraction. In these studies, we find that 2 to 100mM $CaCl_2$ produces a phase separation in EYPE bilayers, where both phases swell as uncharged lipid arrays. We conclude that Ca^{2+} has a higher affinity for EYPE than for EYPC, and that Ca^{2+} induces phase separations in mixed acyl chain phospholipid systems.
This work is supported by NIH grant GM 30115-01.

T-PM-Pos124 MEASUREMENT AND CHARACTERIZATION OF FORCES BETWEEN PHOSPHOLIPID BILAYERS CONTAINING GLYCOLIPIDS. J.M. Collins, K.H. Erpamir*, and L.J. Lis, Department of Physics, IIT, Chicago, Illinois 60616.

Glycosylated segments of cellular lipids and proteins have been hypothesized to be involved in various steps in the approach of membrane surfaces leading to adhesion. We have studied lipid mixtures containing bovine brain cerebrosides (mainly hydroxy acyl chains) and dioleoylphosphatidylcholine (DOPC). X-ray diffraction results indicated that only one lamellar phase was present in the lipid mixtures studied at full hydration. The osmotic pressure technique of LeNeveu, et al., (Nature (1976) 259: 601-603) was then used to measure the net repulsive force as a function of bilayer separation for the lipid mixtures. This net repulsive force was divided into a hydration force (Lis, et al., Biophys. J. (1982) 37: 657-672), electrostatic force (Lis, et al., Biochemistry (1981) 20: 1771-1777) and the van der Waals force for DOPC bilayer alone. Our data consistently yielded an increase of the Hamaker constant in the van der Waals attractive force expression by three orders of magnitude for the cerebroside-DOPC bilayers as compared to DOPC bilayers. We will compare these results with similar work on DOPC-cerebrosides (primarily non-hydroxy acyl chains) and DOPC-ganglioside systems presently underway in this laboratory.
(The latter stages of this study are being supported by NIH grant GM 30115-01)

T-PM-Pos125 EFFECT OF PRESSURE ON PHOSPHOLIPID DISPERSIONS.

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We have investigated the pressure-dependent polymorphic phase behavior of aqueous phospholipid dispersions at constant temperature (30°C) up to hydrostatic pressures of 8.1 kbar (8100 atm). The pressure-induced changes observed in the Raman spectra of dimyristoyl (DMPC), dipalmitoyl (DPPC) and distearoyl phosphatidylcholine (DSPC) are discussed in detail and compared with the temperature-induced structural changes occurring in the same systems. At ambient pressure and at 30°C only DMPC is in the liquid crystalline phase, whereas DPPC and DSPC are already in the gel phase. An increase in pressure must be compared with a decrease in temperature. The gel to liquid crystalline phase transition of DMPC is observed at 150 bar at 30°C. At pressures above 1 kbar a number of gel-gel phase transitions are observed in DMPC, DPPC and DSPC. It is shown that the large angle reorientational fluctuations about the long axes of the acyl chains decrease with increasing pressure.

T-PM-Pos126 INVERTED MICELLAR STRUCTURES IN BILAYER MEMBRANES: FORMATION RATES & HALF-LIVES.

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"Lipidic particle" images in freeze-fracture electron micrographs of bilayers may be either spherical inclusions in the bilayer or grommet-like attachments between bilayers (1). Many have argued that the former would represent inverted micelles enveloped by the monolayer leaflets of a single bilayer (LIPs, e.g., 2) and the latter an inverted micellar intermediate (IMI) in the fusion of apposed bilayers or remnants of these intermediates (e.g., 3). Here expressions are derived for the formation rate and half-lives of both of these structures and the rate of fusion of unilamellar bilayer vesicles via an IMI mechanism. Critical variables are the bilayer lateral compressibility, H_{II} phase dimensions, proximity to the lamellar-to-H_{II} phase transition temperature T_H, energetic barrier to close-apposition (<1 nm) of the bilayers, and (in mixed systems) local mole fraction of H_{II}-forming component. Results computed for egg phosphatidylethanolamine near T_H and pH 7 are in agreement with observation. The LIP formation rate is very slow (a few/μm^2/hr) in unilamellar structures, as observed (3). However, the small vesicle fusion rate is rapid (sufficient to fuse a dispersion in minutes), although not as rapid as in the Ca^{+2}-phosphatidylserine system (4). These calculations show that an inverted micellar model of LIPs and IMI is reasonable, and imply that many LIP-like images in multi-lamellar preparations may in fact be remnants of IMI. (1) Nature 287 166 (1980). (2) Nature 279 162 (1979). (3) Arch. B. B. 207 227 (1981). (4) BBA 688 275 (1982).

T-PM-Pos127 EFFECT OF Ca^{2+} ON THE PHASE TRANSITION PROPERTIES OF A NEUTRAL PHOSPHOLIPID. T.G. Conley and R.L. Biltonen, Dept. of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908.

The effects of Ca^{2+} on the phase transition properties of multilamellar vesicles prepared from dipalmitoylphosphatidylcholine (DPPC) were investigated using high sensitivity differential scanning calorimetry. Increasing the concentration of Ca^{2+} results in both the pretransition and main transition being shifted to higher temperature, with the pretransition being shifted to a greater extent at a given Ca^{2+} concentration. At approximately 300 mM Ca^{2+} the pretransition and main transition merge to form one peak. The ΔH of this peak is the sum of the ΔH values of the pretransition and main transition, indicating that the structural change that is associated with the pretransition still occurs.

In addition, the rate constants for Ca^{2+} penetration through the bilayers of the DPPC vesicle were measured. When Ca^{2+} is added to performed DPPC vesicles and total equilibration is not allowed to occur, two peaks are seen for the main transition. One peak corresponds to lipid interacting with Ca^{2+}, while the other represents Ca^{2+}-free lipid. By comparing the areas of the two peaks as a function of time, the rate constants for Ca^{2+} penetration were determined for samples held above and below T_m. The half-time for penetration is on the order of hours for samples held above T_m and weeks for samples held below T_m.

T-PM-Pos128 CELL PARTITION AND SURFACE FREE ENERGIES RELATED THROUGH CONTACT ANGLES IN TWO POLYMER PHASE SYSTEMS, Kim Sharp, Depts. of Chemistry and Pathology, Univ. of B.C. Vancouver, Canada.

Aqueous solutions containing Dextran T500 and PEG 6000 above certain critical concentrations form two phase systems which have been used to separate cells on the basis of surface properties by partition between the two phase interface and the top phase, where the partition coefficient K = (# in top/# at interface). Boltzmann distribution calculations predict that for particles > 0.3μm dia., K = 0, since the free energy of adsorption to the interface $\Delta G >> kT$, where ΔG is determined by the liquid-liquid interfacial tension (γ_i) and the difference in the cell surface free energy in each phase ($\Delta\gamma$). The contact angle the interface makes with the cell surface (θ) is related by Young's equation to γ_i and $\Delta\gamma$. A model system was studied in which K for human erythrocytes could be increased from 0.02 to 10 by adding < 1μM PEG-palmitate ester. The free energies of the ester-cell surface binding in each phase were -16.6 and -16.8 kT/molecule. The difference in binding energies corresponds to the energy of transferring palmitic acid between the phases, measured independently as 0.18 kT. To determine $\Delta\gamma$, θ and γ_i were measured as a function of ester concentration. For K = 1, 2 x 10^6 ester molecules/cell are bound, $\Delta\gamma$ was increased by 0.04 kT/molecule bound, ΔG was -1.65 x 10^4 kT/cell and the minimum force necessary to pull the cell into the upper phase was estimated as 8 x 10^{-6} dynes. This data and the reproducible but statistical nature of cell partition indicate that the process can be modelled by ln K = $\Delta G/kT$, with an effective temperature of 5 x 10^6 ^oK. We hypothesize that the much larger energies associated with fluid shear stresses generated during coalescence and settling of the phases, rather than thermal energies, are responsible for partitioning large particles such as cells.

T-PM-Pos129 DIRECT DETERMINATION OF THE CALCIUM PROFILE STRUCTURE FOR DIPALMITOYLLECITHIN BILAYERS UTILIZING NEUTRON DIFFRACTION. L. Herbette, R. McDaniel, C.A. Napolitano, Univ. of Conn. Health Center, Cardiology Division, Farmington, CT 06032; Brookhaven National Laboratory, Upton, NY 11973

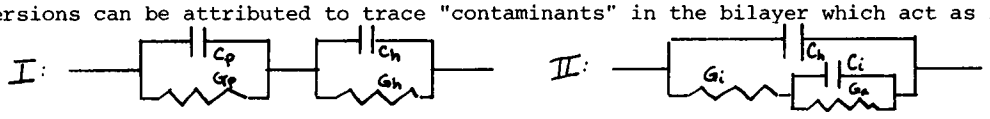
Hydrated oriented multilayers of dipalmityollecithin were prepared containing either calcium-40 ($Ca^{40}Cl$) or calcium-44 ($Ca^{44}Cl$) at a concentration of 5 mM. Lamellar meridional neutron diffraction was recorded from these multilayers on an area detector at the Brookhaven National Laboratory High Flux Beam Reactor. Four diffraction orders were recorded indexing on a repeat of 61$\pm$1 Å. These diffraction patterns were recorded for two relative humidities (66% and 81%) and at 100% H_2O and 10% D_2O. Neutron scattering density profiles were calculated from the background subtracted and appropriately corrected integrated intensity amplitudes which were phased by swelling. The difference profile corresponding to H_2O/D_2O exchange of the same multilayer sample is the water profile structure and was used to scale different samples containing each of the calcium isotopes. A difference profile was calculated for multilayers containing Ca^{40} vs. Ca^{44} in H_2O which corresponds to the calcium profile structure. The water and calcium profile structures were similar at 15 Å resolution showing that both water and calcium are excluded from the hydrocarbon core of the lipid bilayer. The water and calcium profile structures obtained by neutron diffraction were compared to the electron density profile structure at comparable resolution, and both water and calcium penetrate the phospholipid headgroup region of the bilayer profile with the depth of penetration being greater for calcium than for water. We will utilize this approach to locate the high affinity calcium binding sites (1 mole calcium/mole calcium pump protein) of the sarcoplasmic reticulum membrane.

T-PM-Pos130 RAMAN INVESTIGATIONS OF STEROID ANESTHETICS IN DIPALMITOYLPHOSPHATIDYLCHOLINE (DPPC) BILAYERS. Timothy J. O'Leary and Ira W. Levin, Laboratory of Chemical Physics, NIADDK, National Institutes of Health, Bethesda, MD. 20205

The bilayer effects of the steroid anesthetics alphaxalone (3α-hydroxy-5α-pregnane-11, 20 dione, a gift of Glaxo Research, Ltd), progesterone, testosterone, and pregnanedione were investigated by Raman spectroscopy. Polycrystalline samples of DPPC-alphaxalone ranging from 0.1 to 20.0 mole % alphaxalone were prepared by colyophilization from chloroform. A significant interaction between the anesthetic and the lipid headgroup was evidenced by a shift in the headgroup symmetric CN stretching vibration from 711 cm^{-1} (pure anhydrous DPPC) to 720 cm^{-1} (.1 to 10 mole % alphaxalone). Alphaxalone also induces a new spectral line at 860 cm^{-1}, which appears to result from shifting the frequency of an asymmetric CN stretching vibration normally coincident with the 876 cm^{-1} C-C stretching mode. At 20 mole % alphaxalone, a shoulder appears in the 720 cm^{-1} CN stretching feature, which we tentatively attribute to surface inhomogeneity. Small changes in the frequencies of the C-C stretching vibrations at 1062, 1091 and 1128 cm^{-1} may result from indirect effects of headgroup alterations. Liposomes prepared in 70% water at the same anesthetic concentrations show no spectral alterations which are not attributable to the significant lowering and broadening of the phase transition at these high concentrations of anesthetic. This absence of specific interactions is similar to that seen with cholesterol, which only broadens and slightly depresses the gel-liquid crystalline phase transition. The relationship of these effects, and those of the purported alphaxalone antagonist Δ16 alphaxalone, to the mechanism of anesthesia and liposomal stabilization will be discussed.

T-PM-Pos131 AN ALTERNATE INTERPRETATION OF LOW FREQUENCY DIELECTRIC DISPERSIONS IN LIPID BILAYERS. James P. Dilger and Ogden Brandt. Department of Neurobiology and Behavior, SUNY, Stony Brook, NY, and Department of Physics, St. Lawrence University, Canton, NY.

At least 95% of the electrical capacitance of a planar lipid bilayer can be attributed to its hydrocarbon region. Measurements made at frequencies less than 10 Hz, reported by Ashcroft, et al (BBA 643, 191 (81)), reveal the existence of one or more dielectric dispersions. These authors have interpreted the dispersions in terms of circuits like I. In this model, various polar regions of the bilayer (here represented by a single capacitance and conductance, C_p and G_p) give rise to the dispersions. The values obtained for C_p and G_p are reasonable. However, the range of realistic values is large. Circuit I is not unique in generating dispersions of the type observed; Circuit II is also acceptable. Circuit II can be used to model the translocation of lipid soluble ions across lipid bilayers (Ketterer, et al, J. Membrane Biol. 5, 225 (71)). To test whether an ion translocation mechanism would be a possible way to account for the dispersions, we calculated the parameters of circuit II which would generate the observed dispersions. Our results show that the putative lipid soluble ion would be 20xless permeable across the bilayer interior than dipicryl amine, would have a similar interfacial permeability and would be present at an interfacial concentration of one per 20,000 lipids. Thus, we suggest that all or part of the low frequency dielectric dispersions can be attributed to trace "contaminants" in the bilayer which act as lipid soluble ions.



T-PM-Pos132 EFFECTS OF METHYLATION OF PHOSPHATIDYLETHANOLAMINE (PE) ON BILAYER HYDRATION REPULSION AND ON STRUCTURAL TRANSITIONS. N. Fuller, F. Miller, R. P. Rand, Brock Univ., St. Catharines, Ontario and V. A. Parsegian, NIH, Bethesda, MD

Compared to phosphatidylcholine (PC), PE multilayers are considerably less hydrated when equilibrated with excess water. PE bilayers sit much closer together (Biophys. J. 37:657-666, 1982). PE but not PC forms hexagonal phases at high temperature or when dehydrated. These combined effects may account for PE's role in enhancing bilayer fusion. We have sought the reasons for these differences between PC and PE by studying their intermediate species, the singly- or doubly-methylated derivatives of PE as well as by studying mixtures of eggPC and eggPE. We compare phase diagrams, interbilayer hydration repulsion, and structural transitions. The major differences between PE and PC, in bilayer thickness and separation, are also seen between PE and its singly- or doubly-methylated derivatives. A single methylation of PE prevents the appearance of a hexagonal phase at 50°C. Hydration repulsion curves show exponential decays as expected from lipids studied previously. We are analysing the exponentially decaying hydration force for the entire family of phospholipids studied thus far to extract a common decay distance reflecting the properties of water and a coefficient reflecting the polarization of water at the polar group surface. In excess water, lamellar repeat spacings change continuously with PC/PE mole ratio.

	eggPE	$PE(CH_3)_1$	$PE(CH_3)_2$	eggPC
lamellar repeat (A)	52.8	60.0	62.5	62.5
bilayer thickness(A)	32.3	35.5	35.0	35.0
bilayer separation(A)	20.5	24.5	27.5	27.5
lipid area (A^2)	74.7	75.	75.	73.7
phase at 50°C	hexagonal	lamellar	lamellar	lamellar

T-PM-Pos133 CAPACITANCE OF BILAYERS IN THE PRESENCE OF LIPOPHILIC IONS. W.C. Brown and A.D. Pickar. Portland State University, Portland, Oregon 97207.

The capacitance of glycerolmonooleate and egg-phosphatidylcholine bilayer membranes in the presence of NaCl solutions containing tetraphenylborate, tetraphenylarsonium or dipicrylamine ions has been measured using alternating current techniques over a wide range of frequencies. The concentrations of ions correspond to the lower limits of conductance saturation. Similar determinations were also made with solutions containing no lipophilic ions. The experimental method used in this work requires correction of admittance measurements for the solution resistance in series with the membrane, as well as careful area determinations. Drift errors are minimized by making rapid scans over the frequency range (1-200 kHz). In all cases membrane capacitance levels off at sufficiently high frequencies to values which are independent of frequency as predicted by Ketterer, et al. [1]. The high frequency capacitance, which is regarded as the "geometrical capacitance" due to dielectric polarization is practically unaffected by the presence of lipophilic ions. The results support the assumption made in other studies, such as in charge pulse investigations, that the adsorption of lipophilic ions at concentrations up to the saturation range, does not have an important effect on the dielectric properties of bilayers. (Supported by NIH Grant 5R01 ES 937-08.)

[1] Ketterer, B., Neumcke, B., and Läuger, P., J. Memb.Biol. 5 225 (1971).

T-PM-Pos134 THICKNESS-DEPENDENCE OF MONOGLYCERIDE BILAYER CONDUCTANCE. Igor Vodyanoy and James E. Hall. Dept. of Physiology & Biophysics, University of California, Irvine, CA 92717.

Conductance of monoglyceride (squalene) bilayer membranes with different chain lengths (one double bond) was studied (Waldbillig and Szabo, 1979, BBA 597: 455; Vodyanoy and Hall, 1982, Biophys. J. 37: 254a). Current increases faster than linearly with voltage for all membrane thicknesses. Current-voltage curve steepness decreases slightly with increasing membrane thickness. These results are consistent with Neumke and Lauger's prediction (Neumke and Lauger, 1969, Biophys. J. 9: 1160). Zero voltage conductance, G_o, depends strongly on membrane thickness. Temperature dependence of G_o can be described as $G_o = (7.7 \pm 2.02) \cdot 10^5 \exp(- E_i/kT)$, where E_i is the activation energy in a particular monoglyceride membrane, G_o is measured in S/cm^2, and kT has its usual meaning. E itself depends linearly on thickness (regression coefficient 0.967). Our data are summarized in the following table:

Chain length	14:1	16:1	18:1	20:1	22:1
Thickness (nm)	1.97	2.25	2.59	2.86	3.42
G_o (S/cm^2)	$3.1 \cdot 10^{-5}$	$1.9 \cdot 10^{-6}$	$5.4 \cdot 10^{-7}$	$9.3 \cdot 10^{-8}$	$2.2 \cdot 10^{-8}$
E (eV)	0.616	0.633	0.711	0.768	0.797

(Supported by GM 30657 from NIH)

T-PM-Pos135 SOLID STATE NMR STUDIES OF THE BINDING OF URANYL IONS TO DIPALMITOYL PHOSPHATIDYLCHOLINE BILAYERS T.-h. Huang*, A. Blume** and R. G. Griffin, Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, *Department of Physics, University of Maine at Orono, Orono, Maine 04469, and **Institute für physikalische Chemie II, D-7800 Freiburg, Federal Republic of Germany.

^2H, ^{13}C and ^{31}P solid state NMR technique has been used to study the binding of UO_2^{++} ions to specifically labelled dipalmitoyl phosphatidylcholine (DPPC) dispersed in 50 wt % H_2O. At 22°C, addition of more than 0.5 mole ratio of UO_2^{++} completely inhibits the axial diffusion about the molecular axis, as evident from the observations of axially asymmetric ^{13}C powder spectra of breadth characteristic of the immobilized molecule. However, the breadth of the ^{31}P tensor is only about 120 ppm, which is much narrower than that of the pure DPPC, indicating a direct binding of UO_2^{++} ion to the phosphate group. Raising the temperature up to 45°C does not produce any appreciable change in the spectrum. Between 45 and 60°C, the spectra can be simulated with appropriate combinations of an immobilized component and a component which is indistinguishable from that of the liquid crystal spectrum in the absence of UO_2^{++}. Above 60°C, only the liquid crystal component is observed. In all cases when two components are detected, the exchanges between these two components are slow in the NMR time scale. In summary, each UO_2^{++} appear to bind to two DPPC molecules. Binding of UO_2^{++} inhibits the molecular axial diffusion and raises the transition temperature by 17°C. Similar results were observed for DMPC. These NMR results will be compared with our DSC Data.

T-PM-Pos136 A MODEL FOR THE ACTIVATION OF PORCINE PANCREATIC PHOSPHOLIPASE A_2 FOR THE HYDROLYSIS OF DIPALMITOYL PHOSPHATYLCHOLINE LIPOSOMES. By Moshe Menashe, Dov Lichtenberg, and Rodney L. Biltonen, University of Virginia, Departments of Pharmacology and Biochemistry, Jordan Hall, Charlottesville, Virginia 22908.

A model for the activation of phospholipase A_2 for lipid hydrolysis is proposed. This model is based on the observations that, except in the gel-liquid transition region, the enzyme exhibits no activity toward DPPC liposomes. (An exception is with small unilamellar vesicles. In this particular case, these vesicles exhibit surface defects in the gel state and, presumably, these defects provide the impetus for promoting dynamic alteration in structure thus inducing activation of the enzyme.) However, after partial activation of the enzyme within the transition region and temperature-jumping to the liquid crystalline state, maximal activity is immediately observed. The rate of activation is indicated by a "lag" period before rapid hydrolysis occurs which is dependent on enzyme concentrate and inversely dependent upon substrate concentration. These observations lead to the following tentative hypothesis: activation requires the coexistence of structurally distinct regions; the enzyme is most active in the liquid crystalline state; and, the activation process is dependent upon enzyme-enzyme interaction. Thus, we come to the plausible model that the time dependent hydrolysis of lipid is proportional to:

Velocity $\propto$ (Concentration of protein) x (Concentration of substrate)$^{-1}$ x (probability of a structural change).

The details of the various assumptions in the model, their implications, the experimental support for them and some computer simulations will be presented. (Supported by grants from NIH and NSF).

T-PM-Pos137 PHOSPHOLIPID DYNAMICS IN CYTOCHROME b_5 PHOSPHATIDYLCHOLINE BILAYERS. C. Rigell, S. Georghiou and E. Freire Department of Biochemistry, University of Tennessee Knoxville TN 37916.

Pyrene decanoic acid has been used to probe the lateral motion of phospholipids in DMPC and DPPC bilayers containing cytochrome b_5. Static fluorescence measurements above the phase transition temperature of the lipid indicates that the ratio of fluorescence intensities of the excimer and monomer bands decreases monotonically upon increasing the cytochrome b_5/lipid molar ratio. Fluorescence lifetime measurements indicate that the excimer decay is characterized by a double exponential function with a short decay time in the 20 nsec range and a long decay time in the 80 nsec range. In the absence of protein the short decay time is maximal at the lipid phase transition temperature. The long decay time was found to be a monotonically decreasing function of temperature and independent of the protein/lipid molar ratio or the phospholipid species. In all cases the amplitudes of the short and long fluorescence lifetimes were of equal magnitude but of opposite sign as predicted by the theory of excimer formation (Birks, et.al., (1963) Proc-Roy. Soc. A. 275, 575). These results, in conjunction with Monte Carlo calculations of lipid lateral diffusion, indicate that, above the lipid phase transition temperature, the presence of protein molecules slows down the collision frequency of the lipid probes and hence the rate of excimer formation. The origin of this effect appears to be related to an increase in the mean number of diffusional steps required for the collision of two lipid probes and not to a reduced jump frequency. Thus, intrinsic membrane proteins, might affect the rate of bimolecular and higher order reactions by acting as obstacles for the free diffusion of other membrane components even in the absence of any major structural perturbation of the lipid bilayer. (Supported by NIH Grant GM-30819).

T-PM-Pos138 PHOSPHOLIPASE A_2 KINETICS ON MODEL PLASMA LIPOPROTEINS. J.B. Massey, A.M. Gotto,Jr., and H.J. Pownall.[2] Baylor College of Medicine and The Methodist Hospital, Houston,TX 77030.

In plasma, the structure and composition of lipoproteins are continually altered by the action of various lipases. To identify the factors that regulate lipolytic activity, we have studied the action of phospholipase A_2 (PLA) (bee venom, C. adamanteus, and porcine pancreas) on model lipoproteins composed of apolipoprotein A-I (apoA-I) and sn-1,2- dimyristoylphosphatidylcholine (DMPC) or its non-hydrolyzable sn-1,2- diether analogue (DMPC-ether). ApoA-I associates with both lipids forming recombinants whose stoichiometries, dimensions and protein structure are indistinguishable. The DMPC-ether complexes have a slightly higher transition temperature and enthalpy of melting. If albumin is present, the addition of a probe lipid (2 mole % of (1-myristoyl-2-[9(1-pyrenyl)nonanoyl] phosphatidylcholine) to the recombinants allows fluorometric detection of enzymatic activity. The ether PC is non-hydrolyzable ; with the incorporation of a fluorescent diester phospholipid into a diether complex, kinetic and equilibrium binding measurements can be made without altering the "quality of the lipid-water interface". PLA_2 completely hydrolyzes all of the phospholipid in the diester complexes to give lipid-free apoA-I. In the diether complex, PLA_2 hydrolyzes only the fluorescent lipid with the structure of the complexes remaining intact. The time course of hydrolysis of MPNPC in the DMPC-ether complexes is a first order process. The kinetic parameters for hydrolysis by these three enzymes were measured. Complementary thermodynamic parameters obtained from equilibrium gel filtration of [^{3}H]DMPC-ether/apoA-I complexes with PLA demonstrate that the enzymes physically associate with the recombinants. This strategy permits the determination of the rate limiting steps in the process without using preconceived models.

T-PM-Pos139 NMR AND ENZYMATIC STUDIES OF BILE SALT MIXED MICELLES

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Parallel studies of enzymatic accessibility and ^{1}H NMR characteristics have been conducted in a series of taurocholate - lecithin (TC-L) mixtures. By systematically varying the component mole fractions, total lipid concentration, and added salt, physical characterization is possible for a variety of monomer, micelle, and vesicle species. High resolution 500 MHz ^{1}H NMR spectra and nuclear Overhauser effects have been used to study (a) trends in local mobility as a function of overall aggregate size, (b) site-specific TC-L interactions, and (c) equilibria between micellar and vesicle assemblies. Kinetic assays of phospholipase-A_2 and pancreatic lipase/colipase activity show a dramatic sensitivity to the presence of bilayer or mixed micelle substrates, thus providing an additional structural probe in TC-L systems. As expected, these lipolytic enzymes are inhibited by addition of excess bile salt; the effects have been assessed quantitatively in terms of current theoretical models for the action of surface-active enzymes.

T-PM-Pos140 A CALORIMETRIC INVESTIGATION OF THE PHASE PARTITIONING OF THE FLUORESCENT CARBOCYANINE PROBES IN PHOSPHATIDYLCHOLINE BILAYERS. M.F. Ethier[1], D.E. Wolf[2] and D.L. Melchior[1]. [1]UMass Med Center Worcester, MA and [2]Worcester Foundation for Experimental Biology, Shrewsbury, MA.

A number of recent studies have used the selective partition of amphiphiles between lipid environments to probe the lateral domain organization of membranes. Klausner and Wolf [Biochemistry 19, 6199 (1980)] for instance have shown that the fluorescent 1-1'-diacyl-3,3,3',3' tetramethyl indocarbocyanine ($C_N$diI) probes partition between fluid and gel regions of the disaturated phosphatidyl cholines as a function of their acyl chain length. Phase partition preference of the probe was predicted on the basis of quenching due to self association and perturbation of bilayer melting temperature. Determination of partition by elevation or depression of phase transition temperature assumes that the probe does not alter the enthalpy of melt ΔH. To test this assumption and to further define the selective partition of the $C_N$diI's we have measured the effect of these probes on the phase transition of the dimyristoyl (DM), dipalmitoyl (DP) and distearoryl (DS) phosphatidyl choline (PC). At 5 mol % all of the $C_N$diI's altered, in most cases elevating, ΔH. The dependence of ΔH on N systematically shifted with increasing lipid acyl chain length, the largest elevations in ΔH over that observed in pure PC were obtained when N was 2 to 3 methylenes longer than the lipid's chain length. Information about the phase preference of the $C_N$diI's was obtained by considering the relative effects of the probe on the two ends of the transition. Based on these criteria the $C_N$diI's show a chainlength dependent variation in phase preference in each of the PC's, which is largely consistent with fluorescence data.

T-PM-Pos141 WATER PERMEABILITY OF LECITHIN SURFACE BILAYERS. L. Ginsberg†, and N. L. Gershfeld, LPB, NIADDK, National Institutes of Health, Bethesda, MD. 20205.

We have previously reported that surface bilayers form at the equilibrium air-water surface of lecithin dispersions (N.L. Gershfeld and K. Tajima, Nature 279, 708 (1979)). The evidence for surface bilayers was derived from direct measurement of lecithin surface density; at a single temperature the surface concentration spontaneously reached a value exactly twice that of a condensed lecithin monolayer. For dimyristoyl lecithin (DMPC) dispersions the temperature at which surface bilayers form, T_f is approximately 29°. In the present study we demonstrate that the surface bilayer acts as a permeability barrier to water, but only at T_f. Water permeability was obtained from the rate of evaporation of water from the surface of DMPC dispersions in the temperature range 20°-35°. The rate of water evaporation obeys the relation $dw/dt = -DA\ P/x$, where w is the weight of water adsorbed by a detector of area A placed at a distance x above the water surface. P, expressed as concentration, is the difference in vapor pressure at the water surface and at the detector (saturated LiCl), and D is the diffusion coefficient of water in air. Both the DMPC dispersions and pure water obey this relation over the entire experimental temperature interval except near T_f (±0.1°), the temperature of surface bilayer formation, 29°. At this temperature the rate of evaporation from DMPC dispersions decreases by about 50% from that for pure water. Thus, we have demonstrated that surface bilayers act as permeability barriers to water, and that the barrier exists over a very narrow temperature interval of ±0.1° at T_f.

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T-PM-Pos142 IONIZING RADIATION CAUSES DECREASED MEMBRANE FLUIDITY IN ERYTHROCYTE GHOSTS. N.B. Joshi*, C.E. Swenberg, and M.J. McCreery, Radiation Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814. (*NRC Research Associate)

Cellular membranes have been postulated to be important sites for radiation damage. The data reported here on gamma induced alterations in human erythrocyte ghost membranes using the fluorescent probes: 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-anilinonapthalene-8-sulphonate (ANS), suggest that ionizing radiation causes a decrease in membrane fluidity. Hemoglobin free erythrocyte ghosts were prepared from human blood by the procedure of Dodge et al (Arch. Biochem. Biophys. 100, 119, 1963). Test tubes immersed in an ice bath containing the ghost suspension (in 137 mM NaCl, 20 mM Tris-Cl, pH 7.4) were ^{60}Co irradiated with accumulative doses from 500 Rads to 50 kRads. Membrane samples were diluted with buffer to a final protein concentration of 0.45 mg/ml and DPH or ANS was added to the preparation after irradiation (final conc. 20 μM and 10 μM respectively). Fluorescence intensity, polarization and lifetime were measured using an SLM subnanosecond spectrofluorometer and the emission spectrum was recorded with a Perkin-Elmer spectrofluorometer at 25°C. Results indicate that both ANS and DPH fluorescence intensity, F, and the fluorescence polarization, P, increase monotonically with increasing dose. At the highest doses investigated the percentage increase in F for ANS was 7% compared to 20% for DPH, whereas P at 50 kRads increased 4% and 7% compared to unirradiated values for ANS and DPH respectively. The single exponential lifetime of DPH increased with increasing radiation dose, whereas the apparent increase in lifetime of ANS was complicated by multiple components. The increase in fluorescence intensity, polarization and lifetime with increasing doses of gamma irradiation suggest that the membrane fluidity decreases on irradiation. This decrease in membrane fluidity could be due to cross-linkage of lipids.

T-PM-Pos143 MOLECULAR IDENTIFICATION OF PHOSPHATIDYLSERINE AND PHOSPHATIDYLCHOLINE IN PHASE SEPARATED DOMAINS INDUCED BY CALCIUM. S.W. Hui, L.T. Boni, T.P. Stewart and T. Isac, Biophysics Department, Roswell Park Memorial Institute, Buffalo, N.Y. 14263.

Morphological changes associated with calcium-induced phase separation in mixed bovine brain phosphatidylserine (PS) and dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles was investigated by freeze fracture electron microscopy, differential scanning calorimetry, x-ray diffraction, microprobe analysis, and ^{31}P NMR. Dipalmitoyl thionphosphatidylcholine were used in place of DPPC to label PC in ^{31}P NMR and microprobe studies. Up to 25% of PS, there was no detectable morphological segregation induced by 20 mM Ca^{++}. However, there were laterally separated PC- and Ca-rich domains within the same vesicles, and the motion of phosphorus in PS was selectively restricted by Ca^{++}. Lamellar repeat spacing increased with increasing PS percentage. Between 30-50% PS and in excess of 20 mM Ca^{++}, small cochleates were observed to roll out of the surfaces of multilamellar vesicles. The majority of the samples were in the form of small cochleates and small vesicles. X-ray diffraction showed the co-existance of both types of structures. Microprobe analysis showed that calcium and sulfur preferentially associated with cochleates and small vesicles respectively. The results suggested that 30% PS was the critical concentration at which macroscopic structural changes occured. PS was sequestered by calcuim from multilamellar vesicles to form rigid cochleates while the remaining PC was left in the form of small vesicles.

T-PM-Pos144 THE INTERNAL AQUEOUS VOLUME OF SMALL UNILAMELLAR VESICLES CHANGES AT THE PHASE TRANSITION TEMPERATURE OF THE LIPID SHELL, J.L. Slater and T.E. Thompson, Dept. of Biochemistry, University of Virginia, Charlottesville, 22908.

Changes in the fluorescence of partially self-quenched 5(6)-carboxyfluorescein trapped within the internal aqueous compartment of unilamellar phospholipid vesicles can be used to measure volume changes in this compartment. In a previous study we used this method to measure the changes in volume of this compartment caused by the transition of the vesicle lamella from liquid crystalline to gel state in large unilamellar vesicles of dipalmitoylphosphatidylcholine about 700Å in diameter. (D. Lichtenberg et al., Biochim. Biophys. Acta 684, 277-281, 1982). A similar study on small unilamellar vesicles made from this lipid by sonication shows that the volume of the internal aqueous compartment decreases by 34% when the lamellar lipid goes from liquid crystalline to gel. This volume change is completely reversible and is not caused by vesicle-vesicle fusion. It has been shown previously that the Stokes radius of small unilamellar vesicles does not change when the vesicle goes from the liquid crystalline to gel state. However the mandatory increase in bilayer thickness associated with this phase transition cannot be accomodated by a decrease in the radius of the inner monolayer of the bilayer because the lipid in this surface is already close-packed. It therefore seems probable that the decrease in volume of the internal aqueous compartment is caused by a change to a nonspherical shape in the gel phase. (Supported by U.S.P.H.S. Grants GM-14628 and GM-23573).

T-PM-Pos145 THERMOTROPIC BEHAVIOR AND STRUCTURE OF MIXED CHAIN 1,2-DIACYLPHOSPHATIDYLCHOLINES. E.N. Serrallach, G.H. de Haas and G.G. Shipley. Biophysics Institute, Boston Univ. Schl. Med.,Boston, Mass.;Biochemistry Lab.,State Univ. of Utrecht, Utrecht, The Netherlands.

The thermotropic properties and structure of the mixed chain C14/C16 and C16/C14 α-diacylphosphatidylcholines (MPPC and PMPC) were studied as a function of temperature by means of differential scanning calorimetry (DSC) and x-ray diffraction (XRD). After prolonged storage at -3°C, DSC heating scans of MPPC and PMPC show low and high temperature transitions (LTT and HTT) at 26° and 34°C (MPPC) and 13° and 27°C (PMPC). The enthalpies for the LTT and HTT are 8.0 and 8.1 for MPPC and 7.7 and 7.8 Kcal/mol for PMPC respectively. Upon cooling, only the HTT exhibits complete reversibility with full enthalpy. Upon reheating, a broad LTT is observed with ∿ 20% of the original enthalpy followed by the HTT. Prolonged incubation at low temperature is necessary to regain the complete transition enthalpy of the LTT. For both MPPC and PMPC, XRD patterns indicate three different lamellar phases: (a) below LTT, a "crystalline" phase, Lc, with an ordered chain packing, and a lamellar spacing periodicity d = 59Å for MPPC and 60Å for PMPC; (b) between the LTT and HTT, a gel phase Pβ' with hexagonal chain packing and d = 67Å for MPPC and 65Å for PMPC as well as a ripple periodicity of about 200Å; and (c) above the HTT, a liquid crystalline phase, Lα, with d = 64Å for MPPC and 58Å for PMPC. Following rapid cooling from the Lα phase, XRD patterns of samples at -3°C show progressive changes in the wide angle region. The appearance of a sharp line at 4.50Å for MPPC, and a broad peak at 4.45Å for PMPC correlate with the growth in enthalpy of the LTT. Compared to α-DPPC, MPPC and PMPC show no evidence of a "stable" Lβ' phase, and the sub- and pre-transitions appear to merge into the LTT.

T-PM-Pos149 DYNAMIC MORPHOLOGY OF INTERACTING PHOSPHATIDYLSERINE VESICLES. R.P. Rand, Biological Sciences, Brock University, St. Catharines, Canada, B. Kachar & T.S. Reese, NIH, Bethesda, MD, USA.

We are using two complementary techniques to visualize interaction and fusion of phospholipid bilayers. The first is video-enhanced differential interference contrast (or fluorescence) microscopy; the second combines rapid mixing of Ca^{++} or Mg^{++} with phosphatidylserine (PS) vesicles followed by spray freezing and freeze fracture electron microscopy. With light microscopy we can detect vesicles <.5 μm in diameter and even single bilayers. Large, single bilayer vesicles contact but do not stick to each other in 60 mM NaCl, but upon addition of Ca or $MgCl_2$ they form flat areas of contact lasting seconds. Aggregates of contacting vesicles suddenly collapse as breaks occur at their diaphragmatic areas of contact and at nondiaphragmatic regions of vesicles. Even single vesicles can break. Breaks are complete in less than one video frame (34 msec), but remnants of diaphragms left at breaks between contacting vesicles may last several seconds. For freeze fracture, single bilayer vesicles were frozen before, or ≤100 ms, 30 sec, or several min after rapid mixing with 5 mM $CaCl_2$. Single vesicles, doublets, and triplets from early times as well as large, collapsed multilamellar aggregates from late times are made up of smooth bilayers. However, at 30 sec, when the fusion rate is reported to be maximum, fracture faces have ridges and grooves with high curvatures (10 nm dia.) which could either represent collapse of small vesicles, the remains of broken diaphragms, highly folded bilayers, or even cylindrical micelles. Two issues are under investigation: Are there lipid compositions and aqueous conditions where vesicle contact leads to preferential breaking of the diaphragm at contact points (i.e. proximity focused breaks)? What is the structure and role of the high curvature deformations which appear during peak periods of bilayer interactions?

T-PM-Pos150 LIPOSOME-MEDIATED TRANSFER OF MACROMOLECULES INTO FLAGELLATED CELL ENVELOPES FROM *ESCHERICHIA COLI*. Michael Eisenbach, Laurence Klein, and Peter I. Lelkes, Dept. of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel.

Towards a future goal to insert chemotaxis proteins into a subcellular system of flagellated cell envelopes (CE) from bacteria, we studied the interaction between these envelopes and liposomes. Oligolamellar liposomes, composed of 7:1:2 azolectin : phosphatydil serine : cholesterol, were prepared by freezing and thawing and sized to 0.4 μm. These liposomes were chosen due to their high entrapment capacity and low leakiness. Liposomes:CE interaction was monitored by a filtration assay with double-labeled liposomes containing [^{3}H]inulin and [^{14}C]DPPC. Under optimized incubation conditions at least 4% of both labels were found in association with the CE after extensive washing and chasing. The nature of the liposome:CE interaction was studied fluorometrically by monitoring the kinetics and the extent of transfer of liposome-encapsulated carboxyfluorescein (CF) into the CE. In line with our radio-tracer studies, 4-12% of the liposome content could be transferred into the CE. Similarly, electron-microscopic observations suggest that liposome-contained ferritin was found within the CE interia. We conclude that liposomes may be used as vectorial carriers to introduce, probably by fusion, their contents into CE. (Supported by research grants from the U.S. National Institute of Allergy and Infection of Diseases and from the U.S.-Israel Binational Science Foundation (BSF), Jerusalem, Israel. M.E. holds the Barecha Foundation Career Development Chair.)

T-PM-Pos151 GIANT LIPOSOME FORMATION BY FREEZING AND THAWING IN THE PRESENCE OF VARIOUS SOLUTES, FOLLOWED BY DIALYSIS AGAINST HYPOTONIC MEDIA
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Giant vesicles (diameters greater than 0.01 mm) were prepared by freezing and thawing sonicated vesicles in a very high solute concentration followed by dialysis against a lower concentration of electrolytes to expand liposomes by osmotic inflation. Giant liposome formation was monitored by measuring the encapsulated volume and by directly counting the large vesicles under the microscope. Several significant effects of solute on the liposome formation were observed: (1) The resultant liposomes had large trapped volumes if the liposomes were frozen and thawed in high (>1 M) or low (a few mM) but not intermediate concentrations of electrolytes. In spite of the significant trapped volume of liposomes generated in the low concentration case, practically none of these vesicles had diameters larger than a few microns. (2) When high concentrations of KCl or RbCl were present during freezing and thawing, dialysis against a low concentration of electrolyte generated giant vesicles. On the other hand, LiCl did not support the generation of giant vesicles at all, and CsCl did so slightly. NaCl was effective only when the lipid concentration was very high. (3) When chaotropic reagents, such as trichloroacetate, thiocyanate, nitrate, guanidine and urea, were present during freezing and thawing, dialysis generated giant vesicles. Trichloroacetate, an extremely chaotropic reagent which can dissolve lecithin, was effective at a concentration as low as 100 mM. (4) The presence of sugar during freezing and thawing inhibited the subsequent formation of large liposomes, although, glycerol led to the formation of small numbers of vesicles up to 0.05 mm in diameter.

T-PM-Pos152 AFFINITIES FOR ERYTHROCYTE/MIXED PC:PS VESICLE AND PC VESICLE/VESICLE AGGREGATION IN DEXTRAN (36,000 MW) SOLUTIONS. E.Evans, Pathology, Univ. of British Columbia, Vancouver, B.C.

Affinities (free energy reduction per unit area of contact) for red cell-vesicle aggregation were derived from measurements of the extent of encapsulation of a vesicle with the use of a relation for affinity based on elastic energy storage in deformation of the red cell membrane. Affinities for vesicle-vesicle aggregation were determined from measurements of the tension induced in an initially flaccid vesicle membrane as it adhered to another vesicle. Both types of experiments involved micromanipulation of red cells and/or vesicles: vesicles were selected from a chamber on the microscope stage which contained the vesicle suspension; transferred to a second chamber which contained either a red cell suspension in dextran or simply the dextran (36,000 MW) salt solution (150 mM); the capsules were then maneuvered into position for contact. Vesicles were formed from mixtures of egg lecithin (PC) and phosphatidyl serine (PS). For (PC:PS) ratios less than 2.6:1, no aggregation with red cells was detected. In the range of (PC:PS) ratios from (4:1) to (2.6:1), the aggregation with red cells only occured within a window of concentrations from 1 to 8 gm % of dextran. The peak affinity for aggregation, derived from the extent of red cell encapsulation of the vesicle, increased with PC content. Values up to 1-2 x 10^{-2} ergs/cm^2 were measured. For pure PC vesicles, the affinity for aggregation with red cells appeared to increase without limit at higher dextran concentrations. For the vesicle-vesicle tests, one vesicle was aspirated with sufficient suction pressure to create a rigid sphere outside the pipet; the other vesicle was allowed to spread over the rigid vesicle surface. The affinities for aggregation of pure PC vesicles, derived from the membrane tension versus contact area, showed a linear increase with concentration of dextran. The values were on the order of 10^{-1} ergs/cm^2 at 10 gm %.

T-PM-Pos154 AGGREGATION AND FUSION OF MEMBRANE VESICLES DURING FREEZING AND THAWING. George Strauss and Suzanne M. Gibson, Dept. of Chemistry Rutgers University, New Brunswick, NJ 08903.

The structural rearrangement of bilayer vesicles during freezing to -70°C and subsequent thawing (F/T) was investigated. Light scattering and electron microscopy were used to determine size changes. Energy transfer between membrane-bound fluorescent probes gave the fraction of vesicles aggregated and/or fused. The probes were cyanine dyes with C_{18} alkyl sustituents, and chlorophyll *a*. Small unilamellar vesicles of ~300-400 Å dia. were transformed into oligolamellar ones of ~1000 Å. The latter were inert upon further F/T treatment. Vesicles formed by injection of an ethanolic lipid solution into aqueous buffer showed greater changes during F/T than those prepared by sonication. Vesicles consisting of negatively charged phosphatidyl serine aggregated to a larger extent than neutral phosphatidyl cholone vesicles. During F/T, solutes such as carboxyfluorescein present in the external solution were trapped in the aqueous vesicle cores, while solutes originally in the cores were lost. A similar equilibration of lipophilic probes between the membranes and the external solution occurred during F/T. The data indicate that F/T causes a transient loss of bilayer stability resulting in extensive inter-bilayer and bilayer-solution exchange of membrane-bound constituents, and of the contents of the vesicle cores. Supported by the Charles and Johanna Busch Bequest.

T-PM-Pos155 AGGREGATION AND FUSION OF UNILAMELLAR VESICLES BY POLYETHYLENE GLYCOL.
L.T. Boni, S.W. Hui, Biophysics Dept., Roswell Park Memorial Institute, Buffalo, NY 14263, and John T. Ho, P. Mukherjee, Physics Dept., State Univ. of New York at Buffalo, NY 14260.

The interaction of the fusogen, polyethylene glycol (PEG), with small unilamellar vesicles (SUV) of egg phosphatidylcholine (PC), bovine brain phosphatidylserine (PS) and dimyristoylphosphatidylcholine (DMPC) were studied by dynamic light scattering, freeze fracture and negative stain electron microscopy, and ^{31}P and ^{1}H NMR. PEG (mw. 6000) at concentrations under 12% is found to induce reversible aggregation of egg PC SUV. Extensive fusion to multilamellar vesicles occurs above 25% PEG, without the necessity of the removal of PEG. Lower molecular weight PEG are less efficient with respect to both aggregation and fusion. Dehydrating agents, such as dextran, glycerol and sucrose, do not induce fusion. NMR results reveal a restriction in the phospholipid motion by PEG greater than what would be expected due to viscosity, aggregation and dehydration. Charge neutralization by the addition of 100 mM sodium is necessary for the aggretion and fusion of PS SUV. Fusion is greatly enhanced below the phase transition for DMPC, with extensive fusion occurring below 6% PEG. The results suggest that the "rigidization" of the phospholipid molecules facilitates fusion, possibly through the creation of defects along domain boundaries. Vesicle aggregation caused by dehydration and surface charge neutralization is a necessary but not a sufficient condition for fusion.

T-PM-Pos156 COMPARISON OF FLUORESCENCE-BASED REACTIONS TO MONITOR LIPID VESICLE LYSIS AND/OR FUSION. Debra A. Kendall and Robert C. MacDonald. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201.

Calcium treatment causes the aqueous compartments of phosphatidylserine (PS) vesicles to communicate with each other. Since lysis also ensues, this communication could occur via the external phase or within the partially restricted confines of aggregates of fragmented vesicles, as well as by fusion. We have used several different assays to characterize the lytic process. With regard to rate, the assays appear to fit a pattern in that those based upon the combination of two components reveal lysis to proceed faster than do those based upon dilution of internal contents. Calcein, calcein-cobalt complex, and methylumbelliferylphosphate (Kendall & MacDonald, J.Biol.Chem., Dec. 1982), as well as dipicolinic acid (DPA), are released rapidly from PS vesicles by calcium, as measured by their reaction with cobalt ion, EDTA, water (catalyzed by phosphatase) and terbium ion (Tb), respectively. In contrast, release of carboxyfluorescein and calcein from self-quenched concentrations, which involves diffusion only, is quite slow. However, Tb release into DPA solution involves combination but reveals slow lysis. Possibly relevant to this exception is that Tb blocks calcium-induced release of carboxyfluorescein and of umbelliferylphosphate when it is present externally and internally, respectively. Other observations are: 1. Millimolar EDTA, used to dissociate vesicles, has addititional consequences that may include the sealing of aggregates; interior calcein becomes accessible to exterior cobalt soon after calcium addition, yet the complex so formed is only partly accessible to EDTA added immediately thereafter. 2. When Tb and DPA are used in a fusion assay, some fluorescence may come from the external phase unless it is verified for each set of conditions that external calcium and 0.1 mM EDTA are suffificent to quickly inhibit the reaction.

T-PM-Pos157 FUSION OF ERYTHROCYTE GHOSTS BY ELECTRIC FIELDS: VISUALIZATION OF INTERMEDIATE STAGES AT SPECIFIC TIMES DURING AND AFTER FUSION BY FREEZE-FRACTURE ELECTRON MICROSCOPY. Arthur E. Sowers, American Red Cross Blood Services Laboratories, Bethesda, Maryland 20814.

Numerous reports of electric field-induced fusion between membranes in contact with each other have appeared in the literature. Fusion is initiated simultaneously in a large fraction of these membranes by applying a relatively high strength direct current pulse. Membranes not in contact with each other (i.e. in suspension) can be easily brought into contact (i.e. in the form of one-dimensional arrays of spherical elements) by exposing the suspensions to an appropriate low strength alternating current field for a short time before applying the high strength pulse (see J.C.B. 95, 240a (1982) and for reviews see J. Memb. Biol. 67, 165 (1982), and T.I.B.S. 7,199 (1982)). Our studies involved the use of ghost membranes and the author's event-coordinated, quick-freezing procedure (P.N.A.S. 78, 6246 (1981)) to precisely control the interval between the application of the fusion-inducing pulse and sample freezing. Preliminary results on ghosts in very low ionic strength media indicate that within 0.5 sec after the fusion pulse, a) extensive vesiculation is complete and takes place inside ghosts with unfused free edges but not in fused membranes or membranes without free edges, b) up to six spherical ghosts can be fused to form one contiguous entity, and c) large holes in the ghosts with free edges have a curled lip with a curvature reversed from that of the original membrane sphere. The latter observation suggests that, in this system, at least one vesiculation mechanism may involve a stress-relieving process at an origin which coincides with free membrane edges rather than de novo budding at sites away from free membrane edges.

T-PM-Pos158 ROLE OF DIVALENT CATIONS IN MEMBRANE FUSION. W.D. Niles[1], M. Eisenberg[2], and A. Finkelstein[1]. [1]Dept. Physiology, Albert Einstein Coll. of Med., Bronx, NY and [2]Dept. Pharmacologic. Sciences, SUNY-Stony Brook, Stony Brook, NY

A possible role of divalent cations in the mediation of membrane fusion is the induction of membrane-membrane aggregation. We have examined the role of divalent cations in the adsorption of phospholipid vesicles to a single planar phospholipid bilayer. The number of multilamellar vesicles (MLVs), containing the membrane impermeant, water soluble fluorescent dye 6-carboxy-fluorescein (6-CF), was detected in a small volume of solution on one side (the cis-side) of a planar asolectin bilayer by a custom-built fluorimeter. MLVs were added to the cis-side, the fluorescence signal (F) was allowed to reach a constant value, and the unadsorbed MLVs were perfused out with MLV-free buffer. A residual F from the bilayer was detected only when calcium or magnesium was present in millimolar levels after MLVs were added. We interpret this F to originate from MLVs bound to the bilayer in a "prefusion" state, since breaking the planar bilayer or lysing adsorbed MLVs with distilled water caused F to vanish. Free 6-CF and/or divalent cations alone in the absence of MLV resulted in no residual F. Moreover, no F signal was detected when divalent cations were omitted from the cis-side during either MLV addition or perfusion. For this system, we propose that the role of divalent cations in membrane fusion is the maintenance of a "prefusion" state, in which the vesicle is bound to the planar phospholipid bilayer.

T-PM-Pos159 FUSION OF VESICLES WITH PLANAR LIPID BIMOLECULAR MEMBRANES. STEADY-STATE AND KINETIC MODELS. Vitaly Vodyanoy* and Randall B. Murphy, Department of Chemistry and Radiation and Solid State Laboratory, New York University, New York, NY 10003

A model which is based on a simple modification of an absorption isotherm has been applied to the process of vesicle-lipid bimolecular membrane fusion. A possible mechanism of the fusion of a vesicle with a membrane can be schematically represented in two stages; vesicle adsorption onto the membrane surface, and coalescence with the bimolecular membrane structure.
If we assume that (1) the conductance of the bimolecular membrane and vesicle are determined by the number of structural defects present, (2) adsorption of the vesicles is determined by the same defects which provide the conductance of the bimolecular lipid membrane, and (3) the adsorption of the vesicles onto the bimolecular lipid membrane results in the incorporation of the defects, as well as the addition of further adsorption sites, a theoretical model which can account for both the kinetic and steady-state fusion behavior can be proposed. We have utilized this model to analyze data on the functional incorporation of vesicles formed from rat olfactory epithelium membranes into planar artificial lipid bimolecular membranes (Vodyanoy and Murphy, Biophys. J. 37, 65a (1982) ; Biochem. Biophys. Acta. 687, 189 (1982)). These data are shown to be consistent with the present theoretical model.(Supported by NBS NB80NADA0007, NSF BNS-8118761)

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T-PM-Pos160 Liposome Mediated Delivery of a Monoclonal Antibody Against Intermediate Filaments to Drosophila melanogaster Tissue Culture Cells. M.F. Walter, P. Uster and D.W. Deamer, Dept. of Zoology, University of California, Davis, CA 95616.

Liposomes provide an important tool for delivery of biologically active compounds such as chemotherapeutic drugs and viral DNA to target cells. It would also be useful to deliver specific antibodies in this manner, and we have investigated methods for delivery of a monoclonal antibody (Ah3) to the Drosophila Kc cell line. The antibody is directed against components of the cytoskeleton, and this permitted us to monitor delivery by immunofluorescence. We established an optimal delivery system by encapsulating the self-quenching dye 5,6-carboxyfluorescein (6CF) in liposomes produced from a variety of lipid mixtures and then monitoring delivery under several fusogenic conditions. Binding of liposomes was enhanced by acidic phospholipids such as phosphatidylserine (PS) and cardiolipin (CL). Fluorescencence localization was punctate and limited to the periphery of the cells. A remarkable enhancement of delivery was observed if liposomes contained phosphatidylethinolamine (PE) in 1:1 mole ratios with PS or CL and if fusion was triggered by addition of µg quantities of protamine sulfate. Over half of the cells showed a marked increase in diffuse intracellular fluorescence,and freeze-fracture images showed clear fusion events with obvious regions of liposome -plasma membrane continuity. Similar experiments were conducted with the antibody Ah3 encapsulated in delivery competent liposomes. The fluorescent staining pattern of Ah3 delivered to cells by liposomes and triggered by protamins sulfate was identical to the pattern observed under conventional staining methods. This method should facilitate biochemical studies of the effects of antibodies targeted for cytoplasmic structures.

T-PM-Pos161 COLICIN E1 MAKES LARGE, ANION-SELECTIVE CHANNELS IN PLANAR LIPID BILAYERS. Stephen Slatin, Lynn Raymond, Alan Finkelstein, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

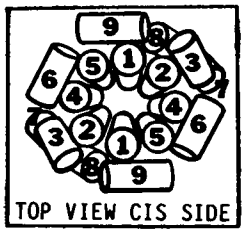
The E1 group (E1, K, Ia, Ib...) of bacterial toxins called colicins are known to form voltage-dependent channels in planar lipid bilayers. The plasmid which codes for colicin E1 has been cloned and the protein sequenced (Yamada, et al., PNAS 79:2827), making this channel amenable to genetic and chemical manipulations. We find that the E1 channel itself is anion selective (Cl^- over K^+), and that the degree of discrimination against cations is pH dependent. This is seen directly in experiments with membranes that have almost no net negative charge. In membranes made of asolectin (approx. 20% negatively charged lipids) the conductance is anion selective at pH 4.5, with a reversal potential of 30 mV for a 10-fold activity gradient. However, if the pH is raised symmetrically (i.e., same pH on both sides of the membrane) to 6.0, the reversal potential becomes 35mV cation selective, much like colicin K under similar conditions (Schein et al., Nature 276:159). Under conditions of asymmetric pH the observed selectivity falls between the values expected for the the different pH's. If the surface charge of the asolectin membrane is screened with high levels of Ca^{++}, the conductance is anion selective below pH 8. These large changes in selectivity occur over a pH range where the single channel conductance varies by less than a factor of 2. In addition to K^+, we have studied other monovalent cations and found that the anion selectivity (using Cl^-as the anion) increases with the size of the cation. However, the channel still shows less than ideal selectivity against cations as large as glucosamine and choline, indicating that the lumen must have an effective diameter of at least 8 Å. (Supported by NIH Grant GM 29210 and 5T32GM7288 from NIG MS.)

T-PM-Pos162 COMPARISON OF THE MACROSCOPIC AND SINGLE CHANNEL CONDUCTANCE PROPERTIES OF COLICIN E1 AND ITS C-TERMINAL TRYPTIC PEPTIDE. F.S. Cohen, J.O. Bullock, W.A. Cramer+, and J.R. Dankert+, Dept. of Physiology, Rush Medical College, Chicago, IL 60612 and Dept. of Biological Sciences+, Purdue University, W. Lafayette, IN 47907.

Colicin E1 is one of a group of colicins that depolarize the E. coli inner membrane and form channels in artificial planar membranes. Two proteolytic C-terminal peptide fragments, with M_r = 18-20 kD, have previously been shown to have depolarizing and apparent channel activity on several membrane vesicle preparations. A comparison has been made in this work of the conductance properties in planar membranes of colicin E1 and a 187 residue C-terminal tryptic fragment. The conductance of both polypeptides, but not their ability to bind to the membrane, is voltage-dependent, with macroscopic current increasing and single channels opening only in the presence of trans-negative voltages, typically -30- -70 mV. The average single-channel conductance of colicin E1 and the C-terminal fragment is 20.9 ± 3.9 pS and 19.1 ± 2.9 pS, respectively. Both molecules require negatively charged lipids to be expressed, have an activity dependence on pH that is pH 5 > 6 > 7, rectify the current to a small extent, and show the same selectivity for Na^+ over Cl^-. It is concluded that the N-terminal two-thirds of the colicin E1 molecule does not modify its channel conductance, nor several properties associated with the channel or channel formation, and that the macroscopic and single channel properties of colicin E1 and its C-terminal tryptic fragment are very similar. (Supported by NIH grants GM-27367 and GM-18457).

T-PM-Pos163 A MODEL OF COLICIN E1 MEMBRANE CHANNEL PROTEIN STRUCTURE. H. Robert Guy, NINCDS, NIH, Bethesda, Md. 20205.

The C-terminal portion of colicin E1, an antimicrobial protein, forms voltage-dependent channels in lipid bilayers. The model shown below is based on the sequence of colicin E1 from residue 355 to the C terminal as determined by Yamada et al.(PNAS 79: 2827). In the model, the channel is a dimer. Each monomer has six transmembrane alpha helices and three shorter helices that surround the channel on the cis membrane surface. Predictions of the helices and their positions in the model are based primarily on calculations of the energy required to move the amino acid side chains from water to either a protein or lipid environment and formation of salt bridges. The inner transmembrane helices 1, 2, 4, and 5 (comprised of residues 355-377, 385-406, 422-439, and 442-453 respectively) are amphipathic with polar faces comprised primarily of Asp, Glu, and Lys residues that line the channel, and apolar faces that either favor a protein environmemt (helices 1, 2, and portions of 4) or the lipid phase (portions of helix 4 and helix 5). Each of the 28 carboxyl groups and 26 amine groups that line the channel can form at least two salt bridges. The surface helices 3, 6, and 9 (residues 409-419, 462-471, and 510-520) are also amphipathic with one face that favors water and an opposing face that favors lipid. Each of the outer transmembrane helices 7 and 8 (residues 474-488 and 491-507) has a face that favors the lipid phase and an opposing face that favors protein. The segment connecting helices 1 and 2 is on the trans side of the membrane and contains three Lys residues that may be responsible for the voltage dependency of channel formation.



T-PM-Pos165 ^{19}F-NMR ANALYSIS OF THE MOTIONS OF M13 COLIPHAGE COAT PROTEIN RECONSTITUTED INTO PHOSPHOLIPID VESICLES. H.D. Dettman, J.H. Weiner and B.D. Sykes, Department of Biochemistry and MRC Group on Protein Structure and Function, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

The major coat protein of the filamentous coliphage M13 is an integral membrane protein during infection. Its fifty amino acids span the cytoplasmic membrane of the E. coli: the acidic N-terminus is in the periplasmic space while the basic C-terminus is in the cytoplasm. There are three phenylalanines found in the hydrophilic domains of the protein while two tyrosines are in the hydrophobic core. These have been substituted in vivo with m-fluoro-analogs. After incorporation into synthetic phospholipid vesicles, the protein was studied using ^{19}F-NMR.

Motion studies of the protein domains within and external to the lipid bilayer have been done. Temperature studies show that the motion of the m-F-tyrosine (Ftyr) rings are very dependent upon the phase state of the lipid chains while the m-F-phenylalanine (Fphe) side chains are not. The hydrophilic regions, though not influenced by the fluidity of the lipid chains, are not freely moving in solution. The linewidth of the Fphe peak is not much narrower than that of the Ftyr. It seems that the hydrophilic domains are either structured and/or are interacting with the vesicle surface. Quantitation of the frequencies of rotation of the aromatic side chains has been done: calculations were done allowing restricted rotations about the α-β bonds and free rotations about the β-γ bonds. For both the Fphe and Ftyr, it was necessary to include "external" contributions to relaxation. The Ftyr ring seems to have rapid interactions with passing lipid chains; the Fphe calculations require backbone motion and interresidue interaction to be included.

T-PM-Pos166 INTERACTIONS OF SPIN LABELED LOCAL ANESTHETICS WITH RECONSTITUTED ACETYLCHOLINE RECEPTOR MEMBRANES. J.P. Earnest[1], J.F. Ellena[2], L. James[1], M.A. Blazing[2], M.G. McNamee[2], and H.H. Wang[1]. 1-Dept. Biology, Univ. of Calif., Santa Cruz, CA 95064 2-Dept. Biochem. Biophys., Univ. of Calif., Davis, CA 95616

The interactions of a spin-labeled local anesthetic with the acetylcholine receptor in reconstituted membrane vesicles and corresponding receptor-free vesicles have been studied. The local anesthetic, a potent analog of intracaine, is labeled with a nitroxide reporter group at the tertiary amine. The resulting electron spin resonance spectrum thus reflects the interactions of the amphipathic anesthetic with polar membrane moieties. The spectrum of the local anesthetic spin label in reconstituted membranes containing dioleoylphosphatidylcholine (DOPC) and acetylcholine receptor reveals at least two components at pH 6.5, one characteristic of spin labels in a fluid lipid environment and the other characteristic of motionally restricted spin labels. The electron spin resonance spectrum of the local anesthetic spin label in pure DOPC vesicles does not show the motionally restricted component. When the pH of the system is raised to 9.5, above the pK of the spin label (pK 7.3), the motionally restricted component of the reconstituted membrane spectrum is abolished. It is suggested that the motionally restricted component reflects a population of charged local anesthetics which are constrained by the acetylcholine receptor. Preliminary results using vesicles of lipid to protein ratio ranging from 150:1 to 180:1 (mole:mole) indicate that 40-50% of the charged local anesthetic spin labels are constrained by the acetylcholine receptor, indicating a high affinity of the spin label for the protein. The interactions of the local anesthetic in charged and uncharged form with vesicles of varying lipid to protein ratios are examined. Experiments are in progress which will examine the effect of the charge of the spin labeled local anesthetic on the function of the acetylcholine receptor within the DOPC vesicles.

T-PM-Pos167 M13 COAT PROTEIN: A NEW RECONSTITUTION AND MEASUREMENTS OF PARTITIONING IN PHOSPHATIDYLCHOLINE/PHOSPHATIDIC ACID VESICLES IN THE PRESENCE OF CALCIUM AND CADMIUM. Robert Bayer, Section of Biochemistry, Cellular and Molecular Biology, Cornell University, Ithaca, NY 14853.

A reconstitution to incorporate the protein asymmetrically spanning the bilayer has been developed. Using this reconstitution, the partitioning of the coat protein between liquid crystal phase and divalent cation-induced gel phase regions of membranes has been determined using quenching of protein tryptophan fluorescence by spin-labelled phospholipids. In addition, the phase behavior of PA/PC mixtures in the presence of calcium and cadmium has been determined using ESR. Results indicate partial clearing of protein from multivalent cation induced gel phase regions in membranes. (Supported by NIH grant HL-18255)

T-PM-Pos168 RHODOPSIN-PHOSPHOLIPID RECONSTITUTION FROM OCTYL GLUCOSIDE SOLUBILIZED SAMPLES BY A DETERGENT DILUTION TECHNIQUE. M.L. Jackson and B.J. Litman, Dept. of Biochemistry, University of Virginia, Charlottesville, Virginia 22908

The transmembrane protein rhodopsin was reconstituted with egg phosphatidylcholine (PC) by a detergent dilution technique. Column-purified rhodopsin, in octyl glucoside (OG), was added to lyophilized egg PC; this solubilized mixture was diluted dropwise into 50 mM Tris-acetate, pH 7.0 to yield a final OG concentration of 10 mM. In this procedure, residual detergent was removed by dialysis, and the reconstituted membranes were concentrated and subsequently subjected to sucrose density gradient centrifugation. The reconstituted membranes formed one major band whose molar phospholipid:protein ratio was in reasonably good agreement with the phospholipid:protein ratio of the starting mixture. For example, starting ratios of 300:1 and 55:1 produced bands on sucrose gradients with ratios of 248:1 and 59:1, respectively. These results are in contrast to those obtained when an OG dialysis technique was used to prepare rhodopsin-egg PC reconstituted membranes. In these studies the maximum phospholipid:protein ratio that was attainable was about 50:1 (Jackson and Litman (1982) Biochemistry 21, 5601). Hence, in the case of OG, the nonequilibrium dilution technique offers a means of obtaining vesicles with a variable phospholipid:protein ratio, whereas the equilibrium dialysis technique does not. This difference in behavior is attributed to the difference in stability of the OG-rhodopsin-PC micelles relative to the OG-PC micelles. Reconstituted samples, which were prepared by the detergent dilution technique to have phospholipid:protein ratios of 80:1 and 85:1, were unilamellar, with an average diameter of $\sim$700 A, and rhodopsin appeared to be symmetrically arranged with respect to its transbilayer distribution.

T-PM-Pos169 RECONSTITUTION OF SARCOPLASMIC RETICULUM CA^{2+}-ATPASE INTO MEMBRANES OF VARYING LIPID CONTENT. G Meissner, BR Lentz, DR Alford, C Kirkman, M. Hoechli. Departments of Biochemistry and Anatomy, University of North Carolina, Chapel Hill, NC 27514.

A new method is presented for reconstituting active deoxycholate-solubilized Ca^{2+}-ATPase of rabbit skeletal muscle sarcoplasmic reticulum into synthetic, saturated phosphatidylcholine membranes containing 50-750 phospholipids per ATPase. Removal of the detergent by dialysis near or above the main phase transition temperature of the pure lipid resulted in the formation of vesicle-like structures as judged by freeze-fracture electron microscopy. Sucrose gradient fractionation and electron microscopy revealed these preparations to be of heterogeneous composition, especially at lipid/protein ratios above 200/1. In contrast, reconstitution at the pre-transition temperature of the pure lipid favored the formation of sheet-like structures with a lipid and protein content close to that of the detergent solubilized sample. Freeze-fracture electron micrographs revealed the Ca^{2+}-ATPase to be organized in rows corresponding to the typical banded pattern seen in low temperature freeze-fracture micrographs of pure lipid bilayers. Incubation at a temperature 5-6°C above the pure lipid main phase transition caused the sheets to close into vesicles of uniform composition and lacking the well organized arrangement of Ca^{2+}-ATPase seen in the sheets formed at the pre-transition temperature. *Supported by American Heart Association Grant 81698, USPHS Grant AM18687 and NSF Grant PCM 79-22733. BRL is an Established Investigator of the AHA.*

T-PM-Pos170 EFFECT OF PHOSPHATIDYLETHANOLAMINE ON SARCOPLASMIC RETICULUM Ca-ATPASE ACTIVITY IN RECOMBINANTS.
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The effect of phosphatidylethanolamine on the activity of sarcoplasmic reticulum Ca-ATPase was studied in reconstituted systems having varying amounts of PE. Delipidated Ca-ATPase was purified from rabbit muscle sarcoplasmic reticulum following the procedure of Dean and Tanford (J. Biol. Chem. (1977) 252:3551). The purified enzyme and mixtures of soy PE and egg PC were solubilized in octylglucoside and reconstituted vesicles formed by detergent dilution (Racker et al., Arch. Biochem. Biophys. (1979) 198:470). The vesicles were purified by sucrose density centrifugation and analyzed for Ca-ATPase activity. In agreement with other workers (Fernandez et al., Biochim. Biophys. Acta (1980) 599:552), no calcium-independent activity was observed in any of the reconstituted vesicles. Highest activity was observed for vesicles with low (0-20 mole percent) PE content and the activity fell rapidly with high concentration of PE. The reconstituted vesicles were then analyzed for protein-lipid ratio, the final PE:PC ratio, morphology as revealed by freeze-fracture electron microscopy and the tendency of PE to form non-bilayer structures. An attempt is made to interpret the effect of PE on Ca-ATPase as a consequence of the tendency of PE to destabilize the bilayer structure.

T-PM-Pos171 ACTIVATION AND INACTIVATION KINETICS OF TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR IN RECONSTITUTED MEMBRANES. J.W. Walker, C.A. Richardson and M. McNamee (Into. by: J. Crowe). Dept. of Biochem. and Biophys., University of California, Davis, CA 95616.

The kinetics of activation and inactivation of acetylcholine receptor-mediated ion flux in reconstituted membranes has been measured with subsecond time resolution using a quench flow technique. After solubilization in sodium cholate, purification by affinity chromatography, and reconstitution into soybean lipids, the receptor displayed a characteristically fast rate of $^{86}Rb^{+}$ ion influx in the presence of activators. For example, at 4°C, 1 mM carbamoylcholine (Carb) stimulated a fast ($t_{\frac{1}{2}}$ = 7 msec) first-order filling of vesicle internal volume. The concentration dependence was sigmoidal with a half-maximal value at 3 x 10^{-4} M Carb. Carb also induced a two-stage inactivation (desensitization) process of the flux response in the reconstituted membranes. Inactivation was measured by preincubating membranes with Carb for various times before measuring flux rates. The two inactivation processes were each characterized by a distinct maximum rate at 2.5 x 10^{-4} Carb and the fast inactivation was half-maximal at 1.3 x 10^{-3} Carb. The concentration dependence curves for both inactivation processes were approximately hyperbolic. In preliminary studies, the effects of chemical modifications on each phase of receptor activation and inactivation were measured. Reduction of the disulfide groups at the agonist binding sites with dithiothreitol decreased the cooperativity of activation and shifted the activation dose-response curve to higher Carb concentrations. The results will be discussed in terms of models that describe the relationship between ligand binding and the processes of channel activation and inactivation. (Supported by USPHS Grant 13050.)

T-PM-Pos172 FUNCTIONAL RECONSTITUTION OF STRIATAL DOPAMINE RECEPTORS INTO PLANAR LIPID BIMOLECULAR MEMBRANES. Vitaly Vodyanoy and Randall B. Murphy, Department of Chemistry, New York University, New York, NY 10003 and Department of Physiology and Biophysics, University of California, Irvine, CA 92717

Rat striatal membranes were purified and sonicated to obtain vesicles of approximate diameter 1-2 micrometers. These vesicles could be fused with planar, essentially solvent-free lipid bimolecular membranes, prepared according to our previously described methods (Biochem. Biophys. Acta 687, 189-94 (1982)). Membranes containing striatal membrane proteins evidenced a dose-dependent increase in electrical conductance upon the addition of the specific dopaminergic agonists apomorphine and ADTN. These increases could be prevented by pretreatment by 1 micromolar (+)-butaclamol, but not by the (-)-enantiomer. Calculation of a K_d for apomorphine subject to a Langmuir isotherm yielded a value of about 1.5 nM, in excellent agreement with radioligand binding results to similar preparations. Using membranes of phosphatidylethanolamine, we observed single channel fluctuations elicited by the addition of apomorphine. The unitary nature of these events was demonstrated by statistical analysis. Measurement of biionic potentials indicated that the charge carrier of these channels was Cl^{-}. It is thus suggested that striatal dopamine receptors which are not linked to adenylate cyclases may utilize chloride channel modulation as a mechanism for transduction. (Funded by NBS NB80NADA0007, NSF BNS-8118761, NIMH MH-32755)

T-PM-Pos173 TRYPTOPHAN FLUORESCENCE QUENCHING BY ORGANIC MERCURIALS IN BRUSH BORDER MEMBRANE VESICLES DERIVED FROM RENAL CORTEX. A. S. Verkman, J. Seifter and A. K. Solomon, Biophysical Lab. Dept. of Physiology and Biophysics, Harvard Medical School and the Renal Division, Brigham and Womens Hospital, Boston, MA 02115

Renal brush border membrane vesicles (BBMV) were prepared from the rat and dog by homogenizing dissected renal cortex and isolating BBMV by differential centrifugation (Booth and Kenny, Biochem. J. 142:575, 1974). BBMV fluorescence spectra consisted of single tryptophan excitation (290 nm) and emission (330 nm) peaks. Addition of p-chloromercuribenzene sulfonate (pCMBS; 0-20 mM) or $HgCl_2$ (0-100 μM) to BBMV gave a rapid decrease in tryptophan fluorescence (< 1s) followed by a slower (1-5 min), exponential, fluorescence decrease. Incubation of BBMV with increasing concentrations of pCMBS and $HgCl_2$ in 28.5 mM NaCitrate + 100 mM NaCl, pH 7.4, 23°C for 3 hrs resulted in concentration dependent decreases in tryptophan fluorescence with single site K_D = 0.3 mM (pCMBS) and 4 μM ($HgCl_2$); addition of 10 mM mercaptoethanol and an additional 2 hr incubation at 23°C resulted in > 90% reversal of pCMBS or $HgCl_2$ quenching. Analysis of the exponential time course for pCMBS quenching suggested the mechanism: pCMBS + BBMV ⟷ pCMBS-BBMV ⟷ pCMBS-BBMV* with a ~2 mM dissociation constant for the initial, rapid bimolecular binding step and a 0.7/min forward rate constant for the conformational change. The pCMBS-BBMV time course ([pCMBS] = 1.5 mM) was not altered by 60 μM furosemide, 25 μM amiloride, 20 μM phlorizin, 250 mM glucose or 1 M urea. These experiments demonstrate that organic mercurial transport inhibitors bind to a sulfhydryl binding site on BBMV and induce a reversible conformational change in a BBMV membrane protein. Supported by NIH grant HL 14820.

T-PM-Pos174 CATION PERMEABILITY OF BAND 3 IN RECONSTITUTED VESICLES. Michael F. Lukacovic, M.R. Toon and A. K. Solomon, Biophysical Laboratory, Department of Physiology and Biophysics, Harvard Medical School, Boston MA 02115

The SH reagent pCMBS(p-chloromercuribenzene sulfonate) induces a cation leak in human red cells (Knauf and Rothstein, J. Gen. Physiol., 58:211, 1971) and inhibits water transport (Macey and Farmer, BBA 211:104, 1970). It has been suggested (Brown et al Nature, 254:523, 1975) that band 3, the anion transport protein, is responsible for pCMBS inhibition of water flux. We have shown (Solomon et al, Ann. NY Acad. Sci., in press) that NEM(N-ethylmaleimide) does not inhibit the pCMBS effect on water flux and that pCMBS binds to band 3 in NEM treated ghosts and have attributed these effects to the only one of the 6 SH groups on band 3 which does not react with NEM. To determine whether band 3 is responsible for the pCMBS induced cation leak, we have incorporated purified band 3 into phosphatidylcholine vesicles by the method of Lukacovic et al (Biochem. 20, 3145, 1981). ^{86}Rb was used to determine that the K leak flux was at least 7.8×10^{-2} nm K/nm band 3, min, about 2% of the K leak in inside-out vesicles (Sze and Solomon BBA 550: 393, 1979). In 4 expts, the ratio of the flux induced by 0.2 mM pCMBS to control was 1.30 ± 0.10 ($p < 0.02$). No cation leak could be induced by 2 mM NEM (10 min, 23°C); the pCMBS induced leak was reversed almost entirely by 5 mM mercaptoethanol. The preparation contained less than 3.3×10^{-6} molecules of Na,K-ATPase/molecule band 3 (our detection limit). Using data of Karlish and Stein (Ann. NY Acad. Sci, in press) the K leak flux from Na,K-ATPase in our preparation is less than 0.2% of our observed Rb influx. These experiments indicate that a cation leak can be induced by pCMBS interaction with a SH group on band 3. Supported in part by NIH grant HL 14820 and Mass. Heart Assoc. grant 13-401-812.

T-PM-Pos180 ERYTHROCYTES OF ELECTROPHORUS ELECTRICUS ACTIVELY TRANSPORT Na^+ AND K^+. Ann S. Hobbs, NINCDS, NIH, Bethesda, MD 20205

Enzymatic properties of (Na, K)-ATPase from Electrophorus electricus have been widely studied, but little information exists for this species on the active Na and K fluxes linked to the enzyme. Ouabain-sensitive Na and K fluxes were measured in erythrocytes from E. electricus in radioactive tracer studies using ^{86}Rb, and also by determination of changes in intracellular sodium and potassium concentrations. The incubation medium consisted of (mM): NaCl, 160; KCl, 0-10; $MgCl_2$, 1.5; sodium succinate, 10; pH 7.2. Incubations were carried out at 30-32° C. Fresh red cells contained 5 mmoles Na and 126 mmoles K per liter cells, while serum values in this animal were 5 mM K and 156 mM Na. During a 40 min incubation in the presence of 0.1 mM ouabain, internal Na rose to 47 mmol/l. cells, and internal K fell to 73 mmol/l. cells. In the absence of ouabain and presence of 5 mM K, the cells still gained Na, but appeared to reach a steady state at approximately 24 mmol/l. In other experiments, Na-loaded cells could not reduce their internal Na in the absence of external K. The presence of glucose had no effect. Active K uptake, as measured by ^{86}Rb, was 8.7 mmol/l.-hr in one animal, and 20.3 mmol/l.-hr in another. In contrast, fresh human erythrocytes usually have active K uptakes of less than 2 mmol/l.-hr. These results suggest that the red cells of the electric eel have an extremely active Na-K pump which should provide interesting new data for comparison with other species and also with the enzymatic data which has been obtained in this species.

T-PM-Pos181 PHASE BEHAVIOR OF SR CA^{2+}-ATPASE RECOMBINANT MEMBRANES. BR Lentz, KW Clubb, DR Alford, G Meissner, M Hoechli. Depts. of Biochemistry and Anatomy, UNC, Chapel Hill, NC 27514.

Using high-sensitivity differential scanning calorimetry, we have recorded the phase behavior of membranes containing an active Ca^{2+}-ATPase and between 29 and 550 lipids (1,2-dipentadecanoyl-3-sn-phosphatidylcholine) per ATPase. Three classes of excess heat capacity peaks were observed in these samples. A broadened "free lipid" peak occured for samples containing between 550 and 200 lipids per protein (T_m = 33.5, as for the order-disorder transition in pure lipid vesicles). Between 200 and 150 lipids per Ca^{2+}-ATPase a broad shoulder became increasingly apparent in the range of 29-32°C. Below 150 lipids per Ca^{2+}-ATPase, a peak at 26-28°C became increasingly prominent with lower lipid content. The temperature dependence of diphenylhexatriene fluorescence anisotropy revealed a similar pattern of membrane phase behavior. Freeze-fracture electron microscopy revealed homogeneous intramembranous particle distributions below 150 lipids per Ca^{2+}-ATPase. However, increasing membrane lipid content produced noticable free lipid patching. On the basis of these observations, we propose that the Ca^{2+}-ATPase is surrounded by a "lipid annulus" of motionally inhibited lipids molecules that do not contribute to a calorimetrically detectable phase transition. Beyond the annulus, "secondary domains" of disrupted lipid packing account for the peak at 26-28°C and the 29-32°C shoulders. At high lipid to protein ratios, the secondary domains coexist with protein-free patches, which account for the peak at 33.5°C. *Supported by American Heart Association Grant 81698, NSF Grant PCM 79-22733, and USPHS Grant AM18687. BRL is an Established Investigator of the AHA.*

T-PM-Pos182 MULTIPLE ANTIBODY BINDING SITES ON THE β-SUBUNIT OF (NA-K)ATPASE. ROBERT A. FARLEY, CHINH M. TRAN, AND ALICIA MCDONOUGH. DEPT. OF PHYSIOLOGY AND BIOPHYSICS, USC SCHOOL OF MEDICINE, LOS ANGELES, CA 90033

The glycoprotein (β) subunit of renal medulla (Na-K)ATPase was separated from the α-subunit by chromatography in SDS-containing buffers. Antisera against the β-subunit were prepared in rabbits by immunization with either holoenzyme or purified β-subunit. Proteolysis of the β-subunit in SDS-containing buffers was performed using chymotrypsin, S. aureus V8 protease, subtilisin, and papain. The peptide proteolytic fragments were separated by polyacrylamide gel electrophoresis and were covalently attached to DPT-paper. The peptides were identified using either anti-β-subunit antibodies and ^{125}I-labeled Protein A, periodate oxidation followed by $NaB[^3H]_4$ reduction, Coomassie blue, or lactoperoxidase-catalyzed Na $[^{125}I]$ labeling. Several fragments with relative MW between 58,000 and 3,000 were identified. The larger chymotryptic fragments of the β-subunit were all found to contain a common antibody binding site and all are also glycosylated. A second antibody binding site on the β-subunit was detected on proteolytic fragments of the β-subunit generated by V8 protease cleavage. This second site is not present on any of the chymotryptic fragments larger than about 20,000 daltons and lies very close to either the NH_2-terminal or the COOH-terminal of the β-subunit polypeptide. Two different antibody binding sites are detected on chymotryptic fragments of about 153 and 181 amino acids that are resolved on SDS-urea gels. Binding of anti-β-subunit antibodies to any of these sites has no effect on steady state (Na-K)-ATPase activity. (Supported by Amer. Heart Assoc., Greater LA Affiliate, and USPHS).

T-PM-Pos183 **THE INTERACTION OF A MEMBRANE ATP POOL AND THE NA:K PUMP IN HUMAN RED CELL GHOSTS.** David G. Shoemaker and Joseph F. Hoffman, Dept. of Physiology, Yale Univ. Sch. Med., New Haven, CT 06510.

This work evaluates the functional relationship between the membrane pool of ATP and certain Na:K pump properties. Hemoglobin-free, high Na_i (150mM), resealed ghosts prepared (0°C) from fresh erythrocytes by two different methods were found to contain 0.3-0.7μmole ATP/liter ghost water. This ATP was inaccessible to use by glucose (10mM) plus hexokinase (6U/ml) incorporated before resealing. Evidently loading the ATP pool via membrane bound phosphoglycerate kinase by incubating with glyceraldehyde-3-phosphate (2mM), ADP(1mM), NAD(4mM), orthophosphate(0.5mM), and diadenosine 5′ pentaphosphate (Ap_5A,0.1mM) markedly stimulated the rate of [^{3}H]-ouabain binding to the ghosts. Glucose and hexokinase incorporated with the glycolytic intermediates had no effect on this stimulation. Neither triose-P, in the presence of hexokinase and glucose, nor ADP plus Ap_5A resealed within ghosts supported significant rates of ouabain binding. In white porous ghosts, the known Na dependent stimulatory effect of ATP on ouabain-sensitive K-pNPPase in the presence of high [Na]:[K] was not elicited by pool ATP loaded via the above glycolytic intermediates, despite being affected by micromolar addition of exogenous ATP. In the absence of Mg, Na and ATP are known to accelerate the rate of [^{3}H]-ouabain dissociation from ghosts. The ATP dependence of this process ($K_{0.5}$=100-500μM) suggests that it is a different one from the ATP effect on K-pNPPase and in turn may be independent of pool ATP. (This work was supported by N.I.H. Grants HL-09906 and AM-17433.)

T-PM-Pos184 FLUORESCENCE LABELING OF THE Na,K ATPase BETA SUBUNIT WITH LUCIFER YELLOW CH. Jonathan A. Lee and P.A. George Fortes, Department of Biology, University of California San Diego, La Jolla California 92093.

Na,K ATPase is composed of one catalytic α subunit and one glycoprotein β subunit of unknown function. To study the β subunit by spectroscopic techniques, we have developed a method to couple fluorescent probes to the carbohydrate portion of the β subunit of purified, membrane-bound, dog kidney Na,K ATPase. The labeling procedure involved oxidation of the carbohydrate in the β subunit with galactose oxidase in the presence of neuraminidase, reaction of the resulting aldehydes with fluorescent hydrazides, and reduction with $NaBH_4$ or $NaCNBH_3$. Lucifer Yellow CH was one of the best labeling reagents tested. After reaction with Na,K ATPase (3 to 20 h @ 0°C) Lucifer labeling stoichiometries ranged from 1 to 5 mol Lucifer/mol ouabain site with <30% loss of ATPase activity. A single fluorescent band corresponding to the β subunit was detected on SDS-PAGE. The excitation and emission maxima of Lucifer-labeled Na,K ATPase (433 nm and 545 nm, respectively) were identical with those of free Lucifer in H_2O and did not change in the presence of Mg+Pi ± ouabain. This indicates that the probe is in an aqueous environment and is insensitive to phosphorylation and ouabain binding. The emission anisotropy was 0.14, indicating motional freedom. The efficiency of energy transfer between Lucifer and anthroylouabain specifically bound to the ouabain site on the α subunit ranged from 5% with 1 mol Lucifer/mol ouabain site to 22% with 5 mols Lucifer/mol ouabain site. Since R_o = 33 A, we estimated an average distance of 54 A between the Lucifer sites and the ouabain site. (Supported by NIH grants HE-20262 and RR-08135).

T-PM-Pos185 EFFECT OF TWO SPHINGOMYELINASE (SPHase) PREPARATIONS ON NaKATPase (E). Edward S. Hyman, Touro Research Institute, New Orleans, Louisiana 70115

Placental SPHase (Sigma) inactivates E as a function of pH, temperature, and time. Incompletely inactivated E (e.g., 120 units SPHase per mg E, pH 6, 30 min, 37° C) has an enhanced k_4 (or $\Delta Pi/E{\cdot}P$) with Na^+ and Mg^{++} approaching k_4 with K^+ added (and may have an increased Pi production per milligram)(Biophys. J., 37, 339a, 1982). Treated E is less affected by Ca^{++} and it is stimulated by 0.1 to 5 mM ouabain (Fed. Proc., 41, #6420, 1982). In contrast comparable units of a purified preparation of placental SPHase (Canadian J. Biochem. 56, 885, 1978; Biochem. J., 195, 373, 1981) has very little effect on the function of E. By two dimensional chromatography the Sigma preparation removed about 44% of SPH, 40% of PC, 15% of CARD, 0% of PS, and 0% of PE, but these values may vary with batches of SPHase and E. The purer preparation removed 56, 23, 11, 0, and 0% respectively. With individual phospholipids, comparable units of the Sigma preparation hydrolyzed SPH as fast as the purer preparation, CARD 2.7 times faster and PC many times faster. The phospholipid content (probably non-essential phospholipid) of an E preparation was also found to vary with that solubilized by detergent treatment in its preparation. In twice the exposure, the Sigma preparation split one-half to two-thirds of the alpha chains of E into two approximately equal fragments (personal communication R. Post). Neither preparation of SPHase is sufficiently specific to study the role of SPH, and neither removes enough SPH to study its role. The effect of SPHases may be compared to the effects of phospholipases C. The major destructive effect of the Sigma preparation is probably attributable to proteolysis, and it may offer an approach to the effect of ouabain. (Supported by the Glazer Reserach Fund)

T-PM-Pos186 LOW ANGLE NEUTRON SCATTERING ANALYSIS OF Na/K-ATPase IN DETERGENT SOLUTION. *+James M. Pachence, *+Benno P. Schoenborn, and +Isodore S. Edelman. +Columbia University and *Brookhaven National Laboratory.

Analysis of low angle neutron scattering from a solution containing detergent solubilized membrane protein provides a direct method to determine molecular weight and radius of gyration of a membrane protein. In this study, a purified Na/K-ATPase preparation from guinea pig outer medulla was first solubilized using the non-ionic detergent Brij 58 (a polyoxyethylene ether) at a weight ratio of 2 mg detergent/mg protein. The purified protein was further delipidated by ion-exchange chromatography on DEAE in the presence of 0.2% Brij 58. The ATPase-Brij complex preserved 30% of the original membrane bound Na/K-ATPase activity at V_{max}. Contrast matching by varying the H_2O/D_2O ratio of the detergent-protein solution was used to eliminate the scattering contribution of the detergent. The scattering intensity at zero angle for a set of proteins of known molecular weight was derived experimentally; the molecular weight of the Na/K-ATPase was thus determined to be 385K-421K by measuring the zero angle scattering intensity from the protein detergent complex at the contrast match point of the detergent. The radius of gyration of the Na/K-ATPase was found to be 76.2 A. The molecular weight and radius of gyration indicate that the Na/K-ATPase is in the dimer form ($\alpha_2\beta_2$) in this micellar preparation.

(This work is supported by NIH AM 31089).

T-PM-Pos187 THE E_1 AND E_2 STATES OF NaK-ATPASE ADOPT SUBSTANTIALLY DIFFERENT CONFORMATIONS. T. Gresalfi and B.A. Wallace; Dept. of Biochemistry, Columbia University, New York, NY 10032

Highly purified Na K-ATPase in membrane patches from guinea pig outer medulla has been examined by circular dichroism spectroscopy. The predominant secondary structure found in the protein is α-helix, similar to many other membrane proteins. The extent of conformational changes in the molecule between the E_1 and E_2 states was examined by placing the membranes in buffers containing high concentrations of Na^+, K^+ or choline (100 mM), or low ionic strength buffers. In the peptide backbone region of the spectrum (190-240 nm), large differences were detected between the $E_1(K^+)$ and $E_2(Na^+)$ states, although virtually no differences were seen between molecules in high and low ionic strength buffers (which produced K^+-like spectra). An extensive conformational change rather than a small local perturbation must give rise to these spectral differences. Using spectra corrected for light scattering and absorption flattening effects, the net number of amino acids involved in this transition has been estimated. These results, when combined with more detailed structure studies, provide information on the mechanism of action of this pump. Supported by NIH Grants AM31089 and GM27292.

T-PM-Pos188 ISOELECTRIC FOCUSSING OF CATALYTIC SUBUNIT OF $(Na^+ + K^+)$-ATPase. Robert L. Post, M. Yamaguchi and S.M. Seiler, Physiol. Dept., Vanderbilt Med. Sch., Nashville, TN 37232

Purified Na,K ion-transport ATPase from outer medulla of pig kidney was first treated with N-ethylmaleimide and then subjected to SDS PAGE according to Laemmli. The catalytic subunit was located in the gel by soaking it in cold 0.5 M KCl. It was excised and subjected to isoelectric focussing on 3.5 percent acrylamide in 2 percent Triton X-100, 9 M urea, and 2 percent Bio-Lyte 3/10 from BioRad Laboratories. At 20°C the isolated polypeptide was resolved into 2 equal bands centered at pH 5.5 about 0.04 pH units apart. The distribution of the polypeptide between the two bands depended on the temperature during focussing. At 15°C predominantly the acidic band and at 25°C predominantly the alkaline band appeared. A temperature jump, midway during focusing, gave a distribution of the polypeptide corresponding to that of the final focusing temperature. Thus the distribution was not a result of covalent modification of the protein. Triton X-165, Nonidet P-40, or Renex 690 could be substituted for Triton X-100. The urea concentration could be 4.5 but not 0 M. Derivatization of the subunit with fluorescein isothiocyanate raised the temperature for an equal distribution of polypeptide between the bands from 20°C to 25°C. The (Ca)ATPase of sarcoplasmic reticulum responded similarly. We tend to think that the catalytic polypeptide retains the intrinsic tendency of the native enzyme to assume one of 2 conformations. Supported by 2R01 HL-01974 from NIH.

T-PM-Pos189 LATERAL DIFFUSION OF CYTOCHROME c IN THE MITOCHONDRIAL INNER MEMBRANE: ROLE OF DIFFUSION IN ELECTRON TRANSFER. Sharmila Gupte, Kenneth Jacobson, Luzia Hoechli and Charles R. Hackenbrock. Laboratories for Cell Biology, Department of Anatomy, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Fluorescence recovery after photobleaching of cytochrome c covalently labeled with fluorescein isothiocyanate (FITC) was determined using calcium fused inner mitochondrial membranes at three ionic strengths to investigate the role of diffusion of this peripheral protein in the electron transfer activity. At low ionic strength (0.3mM Hepes, pH 7.4) more than 90% of the added FITC-cytochrome c was tightly bound to the membrane and the lateral diffusion coefficient (D) was 4×11^{-11} cm^2/sec. When the ionic strength of the buffer was raised to 10mM KPi, D increased to 2.5×10^{-10} cm^2/sec. Further increase in ionic strength to 25mM KPi, resulted in an apparent D of 2×10^{-9} cm^2/sec which fits well with diffusion limited electron transfer by cytochrome c. When 20μm FITC-cytochrome c was added to the membrane followed by washing the percentage of cytochrome c associated with the membrane was lower at higher ionic strengths. Succinate, duroquinol and cytochrome c oxidase activities of the uncoupled membrane showed a 20-50% increase in 10mM KPi compared to 0.3mM Hepes and no further increase above 25mM KPi. These data indicate that a slow diffusion of the membrane-bound cytochrome c at low ionic strength is correlated with a slow electron transfer rate. At higher ionic strengths, there appears to be a dynamic equilibrium between cytochrome c on the surface of the membrane and in the aqueous phase. This dynamic equilibrium between cytochrome c at physiological ionicity may be an important component of diffusion-mediated electron transfer. Supported by NIH GM 28704 and NSF PCM 79-10968 to CRH and NIH GM 29234 to KJ.

T-PM-Pos190 CHARACTERIZATION OF THE Mg-ATPase OF RAT SKELETAL MUSCLE SURFACE MEMBRANE. Troy Beeler, Uniformed Services University of the Health Sciences, Dept. of Biochemistry, 4301 Jones Bridge Road, Bethesda, MD 20814.

Membranes which are believed to be derived from the plasma membrame and/or the transverse tubule were isolated from rat skeletal muscle. While no detectable Ca^{2+},Mg^{2+}-ATPase or Na^{+},K^{+}-ATPase activity was measured in this fraction, it did contain a highly active Mg dependent ATPase (10-20 μmol/mg min). The rate at which ATP is hydrolyzed by this membrane fraction is not linear, but decreases exponetially (first order rate constant $\cong .2$ min^{-1}). The decrease in the ATPase activity depends on the presence of ATP, and is not due to the release of ADP or Pi by the enzyme. Detergents at concentrations which solubilize the membranes inactivate the ATPase; at lower detergent concentrations it appears that they accelerate the rate of ATP dependent inactivation. Once the ATPase is inactivated by ATP, removal of the ATP does not result in reactivation. ATP-dependent inactivation is blocked by Concanavalin A, Wheat Germ Agglutinin and antiserum to the membrane fraction. These proteins do not reverse the inactivation, but prevent further inactivation of the ATPase. Valinomycin, gramicidin, FCCP and A23187 had no effect on the rate of ATP-dependent inactivation. The physiological function of the Mg-ATPase and its inactivation in the presence of ATP is not known. We are now trying to identify which protein is responsible for the ATPase activity and the location of the ATPase within the muscle cell. (Supported by grants from the NIH and ONR.)

T-PM-Pos191 SUBUNIT III OF BEEF HEART CYTOCHROME c OXIDASE IS INVOLVED IN DIMER FORMATION F. Malatesta and G. Georgevich (Introduced by Peter von Hippel), Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Beef heart cytochrome c oxidase is a dimer at physiological pH in membranous vesicles and in non-ionic detergents. We have been able to dissociate the enzyme into monomers by treatment with high concentrations of Triton X100 at pH 8.5 and have established that the monomer is as active as the dimer in electron transfer from cytochrome c to molecular oxygen (G. Georgevich et al., Biochemistry 1983, in press).

Recently we have examined the association of monomers and dimers with regard to which subunits might be involved in this process. We find that enzyme missing subunit III is no longer able to re-associate into dimers. This evidence, coupled with other observations, is taken to indicate that subunit III is in the interface between monomers and provides the M2 domain and C2 subdomain in the model presented by J.F. Deatherage et al., J. Mol. Biol. (1982) 158, 501.

T-PM-Pos192 THE RESPONSE TIME OF THE ΔpH PROBE 9-AMINOACRIDINE DEPENDS ON THE MEMBRANE COMPOSITION.
N.A. Dencher, Dept. Biophysics, Freie Universität, Arnimallee 14, D-1000 Berlin 33,FRG.

The fluorescent amine 9-aminoacridine (9-AA) has been used extensively to measure the proton gradient across biological membranes. This technique is based on the assumption that the uncharged form of 9-AA is freely permeable across the membrane. In order to determine the response time of this method and the parameters which influence it, controlled transmembrane pH gradients were established in about 3 ms by utilizing a rapid-mixing stopped-flow spectrofluorometer, and the induced fluorescence changes of 9-AA were recorded. A variety of reconstituted vesicles were tested. The response time varied from a few to several thousand milliseconds depending on the membrane composition, the temperature, and the presence of KCl and cholesterol. The half-time for the quenching of 9-AA fluorescence following the abrupt formation of a proton gradient of 1.7 pH units across asolectin vesicles was about 110 ms at 38°C and 410 ms at 25°C in the presence of 150 mM KCl. In the absence of KCl a half-time of 270 ms at 25°C was measured. The membrane protein bacteriorhodopsin (asolectin/BR = 49 m/m) was found to increase the half-time about 6 times. In dimyristoyl PC vesicles which undergo a temperature induced gel to liquid-crystalline phase transition the permeation rate of 9-AA is decreased in the gel phase. Experiments done on purple membrane sheets revealed that even in the absence of a pH-gradient 9-AA fluorescence changes occurred upon illumination. The signal is correlated with the formation and decay of a light-induced conformational state of bacteriorhodopsin, i.e. M-411, and is not abolished by addition of ions. This signal has to be taken into account if doing ΔpH-determinations but it can also be used to study the molecular mechanism of this light-driven proton pump.

T-PM-Pos195 FLUORESCENCE STUDIES OF THE INTERACTION OF EQUILENIN WITH LIPOSOMES. Lesley Davenport, Jay R. Knutson and Ludwig Brand; Biology Dept., The Johns Hopkins University, Baltimore, MD 21218.

Equilenin is an estrogen derivative with fluorescence properties similar to naphthol. Since sterols are important constituents of some native membranes, we have studied the interaction of equilenin with single bilayer liposomes using fluorescence. Equilenin was dissolved in ethanol and added to sonicated dimyristoyllecithin vesicles at a 1:500 probe to phospholipid molar ratio. The interaction of the estrogen with the liposome can be observed via an increase in the fluorescence intensity or emission anisotropy with time (2 mins.). With excitation at 336 nm the fluorescence emission anisotropy increased from 0.02 to 0.15 on interaction with the vesicles. When the vesicles are passed down a Sephadex G-25 column, the equilenin fluorescence and scatter due to the liposomes are observed in the same fraction. The steady-state emission anisotropy of equilenin in these vesicles as a function of temperature shows a decrease at the phase transition temperature of DML. Thus equilenin appears to be sensitive to the physical state of the bilayer. The fluorophore is partially accessible to quenching by iodide and may therefore be located near the polar head group region. Equilenin is expected to show excited-state proton transfer as has previously been observed with naphthol. Fluorescence decay studies of equilenin in succinate buffer at pH 5.5 across the emission band are consistent with a two-state excited-state reaction. At 420 nm (on the red edge of the emission), a negative preexponential term is observed, indicating the creation of a deprotonated species during the lifetime of the excited state. Decay associated spectroscopy (DAS) shows a negative emission band in the 420 nm spectral region associated with one of the decay times, as predicted by theory. Equilenin is a semi-native probe whose fluorescence properties depend on pH in a defined way. It may thus be a useful probe for determining the micro- pH at a liposome or protein surface. (Supported by USPHS GM11632. JRK is a fellow of the Pharmaceutical Manufacturers' Association Foundation).

T-PM-Pos196 FLUORESCENCE QUENCHING BY HEMOGLOBIN IN IRRADIATED ERYTHROCYTE GHOSTS. N. B. Joshi*, C. E. Swenberg, and M. J. McCreery, Radiation Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814. (Intro. by D.R. Livengood). (*NRC Research Associate)

Previous fluorescent studies on the effects of ionizing radiation on erythrocyte ghost membranes have yielded inconsistent results. Yonei and Kato (Rad. Res. 75, 531, 1978) and Purohit et al (Int. J. Radiat. Biol., 38, 147, 1980) reported observations which led to very different conclusions by the respective authors as to the change in fluidity and hydrophobicity of these membranes. We report here that these differences can be reconciled by consideration of fluorescence quenching by hemoglobin (Hb) and methemoglobin (MetHb) which may contaminate erythrocyte ghost preparations to varying degrees. Erythrocyte ghosts were prepared by the method of Dodge et al with some modifications which resulted in nearly Hb-free membranes. Control and Hb-enriched (20 g/ml and 125 g/ml) suspensions were then ^{60}Co irradiated with total doses from 1 kRad to 50 kRad. DPH and ANS were respectively added to the exposed and unexposed membrane suspensions and their fluorescence characteristics measured. The addition of Hb reduced both the fluorescence intensity (F) and lifetime of both DPH and ANS. Exposure to ionizing radiation further decreased these spectral parameters. The addition of 125 g/ml Hb produced a 79% reduction in F of DPH compared with control. 50 kRad ^{60}Co further decreased F by an additional 4%. The irradiated Hb-doped ghosts exhibited a blue shift of the soret bands in the absorption spectrum suggesting the production of MetHb. It is concluded that the conflicting reports on radiation damage in erythrocyte ghosts can be resolved on the basis of Hb contamination. In the absence of Hb exposure to ionizing radiation results in an increase in both F and polarization whereas added Hb results in a quenching of F with increasing radiation dose. The quenching of DPH and ANS fluorescence by Hb can be explained in terms of radiative and non-radiative transfer of excitation energy.

T-PM-Pos197 ETHANOL ALTERS MEMBRANE POTENTIAL IN SYNAPTOSOMES AS MEASURED BY RHODAMINE 6G FLUORESCENCE. Preeti Gangola* and Harish C. Pant. LPS/NIAAA, 12501 Washington Avenue, Rockville, MD 20852. The fluorescence of Rhodamine 6G in synaptosome suspension changes markedly when the membrane potential is altered. In the present study we report the effect of ethanol on membrane potential in synaptosomes using the rhodamine 6G fluorescence. This dye has a strong fluorescence in aqueous solution. Upon addition of synaptosomes the fluorescence intensity decreases markedly due to binding of dye to the membrane, which is related to the membrane potential. The fluorescence signal responds to the depolarization produced by increasing concentration of K^+ outside the synaptosomes. The fluorescence intensity increases with increasing $[K^+]_0$ from 5 mM to 60 mM and levels off $\sim$ 80 mM. When the $[Na^+]_0$ concentration was changed from 0 to 137 mM, keeping $[K^+]_0$ constant at 5 mM, no change is observed in fluorescence. On addition of ethanol, the fluorescence intensity increased. There was a significant change (10%) in fluorescence intensity with as low as 10 mM ethanol. The fluorescence intensity increase as ethanol concentration was varied from 10 to 100 mM. Ethanol had no effect on depolarized (137 mM KCl) synaptosomes. The effect of ethanol on membrane depolarization was similar to K^+ depolarization. The membranes were also depolarized by Ca^{2+} (1-200 uM). Patassium was more effective than calcium. In the absence of synaptosomes ethanol had no effect in the fluorescence intensity of dye. In lysed or 12 hr old synaptosomes, there was no change in flourescence either with $[K^+]_0$ or ethanol. We concluded that 1) Rhodamine 6G fluorescence can be used to monitor the change in membrane potential in synaptosomes 2) low doses of ethanol produces changes in membrane potential 3) increasing ethanol concentration produces more membrane depolarization similar to K^+ and Ca^{2+}.

T-PM-Pos198 LATERAL DIFFUSION OF A UBIQUINONE ANALOGUE IN PHOSPHOLIPID BILAYERS AND THE MITOCHONDRIAL INNER MEMBRANE. +En S. Wu, *Chang A. Yu and +Charles R. Hackenbrock, +Laboratories for Cell Biology, Department of Anatomy, University of North Carolina, Chapel Hill, NC 27514 and *Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078.

A ubiquinone analogue, 2,3-dimethoxy-5-methyl-6 (10-hydroxydecyl)-1,4-benzoquinone ($Q_0C_{10}OH$), has been labeled with the fluorescent probe N-4-nitrobenz-2-oxa-1,3-diazole-hexanoic acid (NBDHA). The labeled ubiquinone derivative (Q_0C_{10}-NBD) was reconstituted in phospholipid multibilayers formed of dimyristoylphosphatidylcholine (DMPC) and also incorporated into inner membranes of megamitochondria prepared from cuprizone fed mice. The lateral diffusion of Q_0C_{10}-NBD in these membranes was studied by fluorescence recovery after photobleaching. In DMPC membranes, the lateral diffusion was measured as a function of temperature. Above the liquid-crystal to gel phase transition temperature (Tm), the diffusion coefficient (D) for Q_0C_{10}-NBD was (2±1) x 10^{-8} cm^2/sec. D dropped by 50-fold near Tm. Below the Tm, D was (3±1) x 10^{-10} cm^2/sec. In the mitochondrial inner membranes, the recovery of fluorescence after photobleaching was total, indicating that the incorporated Q_0C_{10}-NBD was completely mobile. The D value for Q_0C_{10}-NBD at 21°C was (2±0.7) x 10^{-9} cm^2/sec, which is about the same as that of lipid diffusion in the mitochondrial membrane. Such a diffusion rate for ubiquinone is consistent with our current understanding of lateral diffusion-mediated electron transfer in the mitochondrial inner membrane. Supported by NIH GM 28704 and NSF PCM-79-10968 to CRH.