W-Pos211 EQUILIBRIUM BINDING OF m-AMSA TO NUCLEIC ACIDS. Rebecca H. Elmore and David E. Graves, Department of Chemistry, University of Mississippi, University, Mississippi 38677.

The potent antitumor antibiotic m-AMSA (4'-(9-acridinylamino)methanesulfon-m-anisidide) (NSC-249992) has been extensively studied due to its ability to interact with nucleic acids. Although the exact antineoplastic activity of m-AMSA remains unknown, recent studies by Wong and Pommier report that m-AMSA may be involved in single-strand scission of DNA. Numerous studies have provided evidence that m-AMSA interacts strongly with DNA, however, these studies have been limited to relatively high drug concentrations and focused on r values greater than 0.1 (r defined as the concentration of bound drug per base pair).

We have adapted UV-visible spectroscopic techniques to examine the interactions of m-AMSA at very low drug concentrations, and provide DNA binding isotherms at much lower r values than have been previously been observed. The studies presented here indicate that m-AMSA binds native calf thymus DNA in a highly cooperative manner under physiological conditions. This positive cooperativity is examined as a function of ionic strength, temperature, and base-pair specificity. These studies are used to determine several thermodynamic parameters of the m-AMSA-DNA complex and provide insight into the drug binding phenomena of cooperativity and binding site selectivity. (This work is supported by the National Cancer Institute, CA-41474).

W-Pos212 PHOTOAFFINITY ANALOG OF ACTINOMYCIN D: A NOVEL PROBE FOR EXAMINING ACTINOMYCIN-DNA INTERACTIONS. Randy M. Wadkins and David E. Graves, Department of Chemistry, University of Mississippi, University, MS 38677.

The interactions of the potent antitumor antibiotic actinomycin D are reversible, thus precluding an indepth investigation of the binding site geometry and site specificity. In an effort to circumvent this reversible nature of binding, we have applied the technique of photoaffinity labeling to study of actinomycin D - DNA interactions. The photoreactive analog (7-azido-actinomycin D) has been synthesized and its chemical properties determined. Studies in which the reversible DNA binding properties are examined reveal that the photoreactive analog retains the thermodynamic and binding specificities of the parent actinomycin molecule (i.e., equilibrium constant, site exclusion size, and GC specificity). However; upon photolysis, the actinomycin analog can be covalently attached in situ to the DNA, allowing the adduct to be recovered and examined in detail. These studies provide a direct approach for examining the binding properties of this antineoplastic agent to DNA through the capacity for covalent modification of the drug-DNA complex. This work is supported through grants from the Cottrell Research Corporation and the National Cancer Institute CA-41474.

W-Pos213 BINDING OF ACTINOMYCIN D AND ETHIDIUM TO SOME OLIGONUCLEOTIDES. D. Jones, E. Ferguson, and F. M. Chen, Department of Chemistry, Tennessee State University, Nashville, Tennessee 37203.

Spectral titrations, melting characteristics and kinetic studies with some oligonucleotides (10-mers) have been carried out in order to elucidate the base sequence specific binding of actinomycin D to DNA. Results from comparative studies with d(ATATGCATAT) and d(TATACGTATA) support the findings that actinomycin D binds strongly to the GC sequence but only weakly to the CG sequence. The binding stoichiometry is found to be one actinomycin D to one duplex d(ATATGCATAT) and the SDS induced dissociation rate of the drug from this DNA is slow enough to be measurable with a conventional UV-Visible spectrometer. Results from studies with other decamers containing contiguous and non-contiguous GC as well as CG sequences are to be presented. Comparison with the kinetic results of poly(dG-dC):poly(dG-dC)-actinomycin D system will also be made. Similar oligonucleotide studies with ethidium bromide do not reveal strong preference for any particular sequence. (Research supported by CA-42682 and a subproject of MBRS Grant S06RR0892)

W-Pos214 COMPARISON OF O^6-METHYLGUANINE BASE PAIRS WITH MISPAIRS.
Barbara L. Gaffney and Roger A. Jones. Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903.

A set of ten non-selfcomplementary nonadeoxyribonucleotides, d(GGTTXTTGG):d(CCAAYAACC), where X and Y include the carcinogenic lesion O^6-methyldeoxyguanine (Gme) and all four normal bases, was synthesized by the cyanoethylphosphoramidite method. From this set 25 different duplexes were characterized by optical melting studies. Thus the stabilities of duplexes containing this modified base opposite all four bases can be compared with the stabilities of oligomers containing all Watson-Crick base pairs as well as with those containing every possible mispair. Furthermore, the effect of nearest neighbors on each of these combinations can be evaluated since the flanking bases are T's in one strand and A's in the other. T_m's of duplexes at $3x10^{-5}$ M total strand in 0.1M NaCl were found to be G:C 45°, C:G 42°, T:A 41°, A:T 38°, G:T 29°, A:G 27°, G:G 27°, T:G 25°, T:T 23°, G:A 22°, T:C 21°, A:C 21°, C:T 19°, A:A 18°, C:A 16°, and C:C 14°. T_m's of duplexes containing the modified base were found to be Gme:C 25°, C:Gme 24°, Gme:G 23°, Gme:T 23°, A:Gme 22°, G:Gme 21°, T:Gme 21°, Gme:A 20°, and Gme:Gme 20°. These results are consistent with our earlier work (*Biochemistry* **1984,** *23,* 5685) in that all duplexes containing Gme 1) are greatly destabilized relative to normal duplexes and 2) are more stable when opposite C rather than T, even though T is preferentially misincorporated during replication. The new results also show that duplexes containing Gme, while less stable than those with G:T mispairs, are nevertheless more stable than those with the least stable mispairs.

This work was supported by grants from NIH (GM31483) and the American Cancer Society (CH248B).

W-Pos215 METAL INDUCED POLYMORPHISM OF DNA. Yong Ae Shin, Patricia Stenger, Marcos Maestre* and Gunther L. Eichhorn. National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224 and *Lawrence Radiation Laboratory, Berkeley, CA.

We have previously observed, by circular dichroism (CD), that the treatment of poly(dGdC)·poly(dGdC) in the B-form with $[Co(NH_3)_6]^{3+}$ leads to a series of conformational changes in the polymer, beginning with the conversion to the Z-form, but continuing with the conversion of Z to other conformers. Such multiple transitions also occur when the polynucleotide conformation is transformed by organic solvents, in the presence (Zacharias et al., JBC 257, 2775, 1982) or absence (Hall & Maestre, Biopolymers 23, 2127, 1984) of metals. We have now demonstrated that metal ions in the absence of other effectors generally are capable of inducing multiple conformational changes in this polynucleotide. The initial B → Z conversion has been studied with various metal ions, which can be placed in the order of decreasing ability to produce Z: Co(III) > Al(III) > Cu(II) > Ni(II), Cd(II) > Zn(II) > Co(II) > Mn(II) >> Mg(II) >> Na^+. This order is that of decreasing stability of the metal complexes, so that the ability of the metals to induce the B → Z transition is correlated with their ability to form bonds to the DNA.

These metals differ in the nature of the further transitions that they produce after Z-formation. Some of the metals, like Co(III), produce an "A" form from Z. Ca(II) and Ba(II) are usually transformed directly from B to "A". The use of fluroscat cells in CD measurements with Co(III), Al(III), Cd(II) and Ca(II) rules out scattering contributions to the CD spectra. The presence of more than one isodichroic point relating a series of spectra (in these cells) with varying ratios of metal to polymer provide evidence for multiple transitions in most cases.

W-Pos216 IN VITRO ANTIVIRAL ACTIVITY INDUCED BY POLY r(A-U) AND INTERCALATING DYES.
James M. Jamison, Pedro J. Bonilla, Daniel G. Flowers, Sutisak Kitareewan, and Chun-che Tsai, Department of Chemistry, Kent State University, Kent, Ohio 44242 and Program in Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Experiments have been designed to systematically examine the effects of intercalating dye induced perturbations on the antiviral activity of poly r(A-U) using the human foreskin fibroblast-vesicular stomatitis virus bioassay system. Dyes that intercalate into the minor groove, major groove, both the minor or major groove of double-helical nucleic acids and whose mode of intercalation is not known have been tested in this study. The concentration of poly r(A-U) was fixed at 0.2 mM (or 0.1 mM), while the dye concentration was fixed at 0.05 mM (or 0.033 mM) to produce a dye/nucleotide ratio of 1:4 (or 1:6). Poly r(A-U) or each dye was tested individually at the concentrations employed in dye/poly r(A-U) combinations. None of the dyes alone or poly r(A-U) alone were efficacious antiviral agents. When poly r(A-U) was combined with the minor groove intercalating dye (ethidium bromide, propidium iodide, adriamycin or daunomycin) or the minor/major groove intercalating dye (9-aminoacridine), the antiviral activity at dye/nucleotide ratios of 1/6 to 1/4 was potentiated 17-fold for ethidium and propidium, 8-fold for adriamycin and daunomycin, and 5-fold for 9-aminoacridine. When poly r(A-U) was combined with other intercalating dyes (carminic acid, chloroquine, quinine, methylene blue or toluidine blue O) or riboflavin or its co-factors (FMN or FAD), antiviral activity at a dye/nucleotide ratio of 1/4 was enhanced 12-fold for carminic acid, 10-fold for chloroquine and quinine, 5-fold for methylene blue and toluidine blue O, and 4 to 8-fold for riboflavin and its co-factors. These results suggest a synergism between the poly r(A-U) and the minor or minor/major groove intercalating dye.

W-Pos217 BASE AND SEQUENCE SPECIFICITY OF THE DAUNOMYCIN-DNA INTERACTION. J. B. Chaires, M. Britt, K. R. Fox and M. J. Waring. Department of Biochemistry, The University of Mississippi Medical Center, Jackson, MS 39216-4505 (JBC and MB) and Department of Pharmacology, Cambridge University, Cambridge CB2 2QH England (KRF and MJW).

Daunomycin is a potent anticancer drug that binds strongly to DNA by intercalation. We report here studies aimed toward the characterization of the base and sequence specificity of its interaction with DNA. Equilibrium binding and competition dialysis studies as a function of DNA base composition show that daunomycin binds preferentially to GC rich DNA. The simplest model to account quantitatively for the experimentally observed dependence of the binding constant on the fractional GC content is one in which daunomycin interacts preferentially with the triplet sequences AGG, GGA or GGG. The method of DNase footprinting was used to map daunomycin binding sites within a 160 b.p. fragment containing the Tyr t promoter, a 61 b.p. fragment from pBR322, and a 53 b.p. fragment from pBR322. The sequence common to all observed protected regions was one containing adjacent GG base pairs flanked by an AT b.p. at the 5' position. The equilibrium binding studies and the DNase footprinting results are mutually consistent. Daunomycin thus preferentially binds to a triplet sequence containing adjacent GC base pairs, flanked by an AT base pair. The same type of binding site was observed in the crystal structure of a daunomycin-oligonucleotide complex, and was predicted to be a specific site in a recent theoretical study (Chen et al. (1986) J. Biomol. Struc. Dynam. 3, 445-466). Supported by Grants CA35635 from the National Cancer Institute (JBC) and by a NSF US-UK Cooperative Science Travel Grant INT-8521004 (JBC).

W-Pos218 SPERMIDINE EFFECTS ON DNA BASE PAIR DYNAMICS: IMINO PROTON EXCHANGE OBSERVED BY NMR. G. Eric Plum and Victor A. Bloomfield, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108

Polyamines are known to stabilize the DNA helix to thermal melting. We are interested in the effects of polyamines on the local stability of the helix at the level of one or a few base pairs and we have used imino proton exchange to study the effects of spermidine on the base pair breathing dynamics of DNA. Poly (dA) - poly (dT) was digested by DNAse I to approximately 100 base pairs. Exchange of the thymine imino proton was observed by nuclear magnetic resonance line broadening. Most experiments were performed at pH 7.0, 100 mM NaCl, 10 mM Na cacodylate and 1 mM EDTA. We find that spermidine is an extremely good catalyst of imino proton exchange. The spermidine base concentrations required to equalize the apparent DNA base pair opening and closing rates are in the micromolar range. Analyzing the data in the way devised by Leroy et al.*, we find that the activation energy for base pair opening is approximately 100 kJ/mole; the dissociation enthalpy is approximately 55 kJ/mole. The apparent base pair lifetime, at 30°C, is about 7 fold greater than that reported for poly (rA) - poly (rU). We find no increase in the apparent base pair lifetime due to spermidine binding when compared to the apparent base pair lifetime determined using 2,4-dimethylimidazole as catalyst. Addition of NaCl, at constant temperature and pH, lessens the catalytic activity of spermidine, presumably by lowering the binding of spermidine to the DNA.

* Leroy,J-L., Broseta, D., and Gueron, M. J. Mol. Biol. (1985) 184, 165-178.

W-Pos219 A FLUORESCENCE PHOTOBLEACHING STUDY OF THE MICROSECOND REORIENTATIONAL MOTIONS OF DNA. Bethe A. Scalettar, Paul R. Selvin, Daniel Axelrod (*), Melvin P. Klein and John E. Hearst, University of California, Berkeley, CA 94720 and (*) University of Michigan, Ann Arbor, MI 48109.

We have conducted a polarized Fluorescence Photobleaching Recovery (FPR) study of the rotational dynamics of ethidium-azide labeled DNA. Polarized photobleaching experiments provide data on microsecond and millisecond molecular reorientation that complement the information available from nanosecond fluorescence depolarization studies.

In polarized FPR experiments an anisotropic angular concentration of fluorophore is created by nonuniformly bleaching dye molecules with a short intense pulse of polarized light. The sample is then weakly illuminated and the temporal variation in the emitted fluorescence is related to molecular reorientation and the associated return of a uniform angular distribution of dye. We have observed, however, that the time dependence of our microsecond FPR curves is determined by more than just the rotational motion of the dye-DNA complex. Hence, to follow reorientational relaxation in our system we conduct FPR experiments in 2 modes (called parallel and perpendicular) that differ only in the polarization of the bleaching light. We then construct a function, r(t), from the data obtained in these two modes; the variation with time of this new quantity is governed solely by processes that are sensitive to the polarization of the incident light (e.g., molecular rotation). We find experimentally that r(t) remains constant for rotationally restricted DNA systems despite a temporal recovery in the parallel and perpendicular FPR curves. We also follow the dynamics of solutions of Lambda DNA as revealed in the temporal dependence of r(t). We find that this DNA system rotationally relaxes after about one hundred microseconds and that the dye-DNA complex reorients substantially during the 10 microsecond bleach period.

This work was supported in part by NIH grant GM30781, DOE grant DE-AC03-76SF00098, and an NSF graduate fellowship.

W-Pos220 BINDING OF THE CARCINOGEN N-ACETOXY-N-ACETYL-2-AMINOFLUORENE TO A DNA FRAGMENT REPRESENTING A SPECIFIC BINDING SITE ON DNA, Stephen A. Winkle, Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903 and Richard S. Sheardy, Department of Chemistry, Penn State University - Hazelton, Hazelton, PA.

Previously we have shown that the carcinogen N-Acetoxy-N-Acetyl-2-Aminofluorene (AAAF) selects for specific regions on pBR322 and ΦX174 [Winkle, et al. Biophys. J. 41, 287 (1983)] and that it further selects certain sequences within these regions [Winkle, et al. Biophys. J. 47, 338a (1985) and Winkle, et al. Biophys J. 49, 50a (1986)]. To gain detailed information on the exact nature of AAAF binding to these sequences, we synthesized using the phosphoramidite method a 16-base pair fragment representative of these sequences:

$$\begin{array}{r} 5'\text{GTCAGCTCTTGCTGCG}^{3'} \\ \text{CAGTCGAGAACG}\overset{*}{\text{A}}\text{CGC} \end{array}$$

^{1}H NMR resonances of the base protons of the fragment were assigned by COSY and 1D-NOE measurements. AAAF was reacted with the fragment to produce one adduct per fragment. NMR studies of the AAF bound fragment indicate that the AAF moiety is bound only to the G indicated (*). NMR melting studies suggest that the bound AAF induces localized distortion of the helix. This is supported by optical Tm measurements (Tm is lowered by 3 C in the presence of the bound AAF) and by CD experiments. NOE experiments of the AAF-bound fragment suggest a possible conformation for the bound adduct. (This work supported by NCI Grant CA 34782 (SAW) and by PSU - FSSF (RDS)).

W-Pos221 THE GEL-SOL TRANSITION IN CONCENTRATED DNA SOLUTION : LOCAL MOTIONS DOMINATE THE P-31 NMR RELAXATION. William H. Braunlin, Michael G. Fried* and Victor A. Bloomfield, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108, and *Department of Biochemistry, University of Texas, Health Science Center, San Antonio, TX 78284.

Intermolecular interactions among DNA molecules are a source of concern for those attempting to interpret the effects of DNA dynamics on the NMR relaxation of nuclei on the DNA. One manifestation of such intermolecular interactions is the gel-sol transition that occurs at typical NMR concentrations of DNA (M. G. Fried and V. A. Bloomfield (1984) Biopolymers 23, 2141-2155). We have examined the consequences of the gel-sol transition on the P-31 NMR relaxation rates of the backbone phosphates of double-helical DNA. Transverse and longitudinal relaxation rates were measured as a function of ionic strength over the course of the temperature-dependent transition. The primary conclusion we are able to draw from these measurements is that the gel-sol transition has little effect on the P-31 NMR relaxation. The implication of this result is that, consistent with previous analyses, it is primarily local motions that influence the P-31 NMR relaxation of the DNA backbone phosphates.

W-Pos222 **TOROIDAL CONDENSATION OF DNA FRAGMENTS: LIGHT SCATTERING AND ELECTRON MICROSCOPY.** An-Zhi Li, Patricia Arscott and Victor A. Bloomfield, Dept. of Biochemistry, University of Minnesota, St. Paul MN 55108

The size and structure of toroids of short fragments of DNA (2700 bp and 1300 bp) condensed by hexaammine cobalt(III) were investigated by light scattering and electron microscopy (EM). There is no significant difference in the size of the toroids produced by the two fragments. EM shows that both have outer radii about 400-450 Å and inner radii about 140 Å, while the Stokes radius from dynamic light scattering is about 450 Å. These values are in the same range observed with monomolecular condensation of high mol wt viral DNA, though about 10 molecules of 2700 bp fragments and 20 of 1300 bp fragments are contained in each toroid. The rate of condensation, measured by light scattering, is approximately first order in concentration of DNA fragments. EM of solutions that had stood for several hours showed clusters of still discrete toroids, connected by a few DNA strands. These results suggest that DNA condensation and aggregation occur through highly structured intermediates, rather than the random aggregates implicit in current theories. A prime theoretical issue is to understand the mechanism of size determination of DNA toroids.

W-Pos223 **LIQUID CRYSTALLINE PHASE TRANSITIONS IN CONCENTRATED SOLUTIONS OF SODIUM DNA.** Teresa E. Strzelecka and Randolph L. Rill, Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306

Aqueous solutions of short DNA fragments (147 bp) in 0.3 M Na^+ buffer separated into isotropic and liquid crystalline (cholesteric) phases when DNA concentration exceeded 120 mg/ml. Transitions between the isotropic and cholesteric phases were monitored using phosphorus-31 solid state NMR spectroscopy. At a given DNA concentration the onset of the isotropic phase upon increase in temperature was indicated by appearance of a sharp isotropic resonance superimposed on a broad resonance of the anisotropic phase. Integrated areas of isotropic and anisotropic resonances were used to quantitatively construct a phase diagram of concentration vs. temperature for transitions between the isotropic and cholesteric phases in DNA solutions from 20^0 to 70^0 C. This phase diagram is in a good qualitative and semi-quantitative agreement with Flory's theory of phase equilibria in solutions of weakly interacting rod-like particles. Specifically, the fully isotropic and cholesteric phase boundaries are only weakly dependent on temperature and are separated by a narrow biphasic region in which the two phases coexist.

Microscopic investigation of samples with DNA concentrations in the range of 130 to 309 mg/ml revealed the presence of at least two different types of mesophases. Cholesteric order was observed in samples with concentrations of 182 to 220 mg/ml and smectic-like phase was observed in samples with concentrations exceeding 250 mg/ml. Transitions between mesophases were reflected by phosphorus-31 resonance linewidth changes with DNA concentration. (Supported by DOE contract EV05888 and NIH grant GM37098.)

W-Pos224 **HEAT INDUCED DNA AGGREGATION IN THE PRESENCE OF DIVALENT METAL IONS.** David A. Knoll, Michael G. Fried* and Victor A.Bloomfield. Dept. of Biochemistry, Univ.of Minn., St.Paul, MN and *Dept. of Biochemistry, Univ. of Texas Health Science Center, San Antonio, TX.

We earlier described the formation of DNA aggregates in the presence of divalent cations at elevated temperatures. We now have measured the effect of DNA molecular weight and anion type on aggregation by determining the fraction of DNA, (1 mg/ml, 0.1M salt) insoluble at a given temperature. Over a range of 75 to 1631 bp we find that the midpoint of the aggregation transition (T_a) is insensitive to DNA molecular weight. In contrast to Mg salts, the anion of Mn salts has a pronounced effect on DNA solubility: $Br^- \approx ClO_4^-$ (T_a=36°C) > Cl^- > CH_3COO^- > SCN^- > $SO_4^=$ (T_a=52°C). Aggregation is only partially reversible (≈50%) upon cooling, but is completely reversible with the addition of EDTA. It has been observed that many divalent metal ions destabilize native DNA under certain conditions, thus raising the possibility that aggregation is the result of strand separation. We have investigated the melting behavior of DNA (50μg/ml in $MnCl_2$) by monitoring absorbance vs. temperature profiles at several wavelengths. While we observe aggregation subsequent to melting at low salt (<10mM Mn), at higher salt we do not see melting, only aggregation at temperatures below the expected T_m. Furthermore, S1 nuclease digestions of redissolved DNA precipitates formed with Mg, Ca, or Mn show no evidence for single strand formation. Therefore we conclude that gross strand separation is not occuring. However, we cannot exclude the possibility that transiently melted regions, which quickly reanneal upon cooling or removal of divalent cation, are responsible for aggregation. A theory of the ion dependence of the helix-coil transition, including site binding to the single-stranded bases, predicts an intermediate range of salt concentrations at which the single-strand form is stabilized, if the linear charge density of the single strands has a suitable value. However, at the high salt of our experiments, the double-stranded form should be more stable.

W-Pos225 **THERMODYNAMIC AND ELECTROSTATIC ANALYSIS OF DNA FLEXIBILITY.** Carl Sunshine and Don Eden, Dept. of Chemistry, San Francisco State Univ., San Francisco, CA 94132.

Using transient electric birefringence, we have measured the rotational decay times for five DNA restriction fragments (184, 192, 213, 234 and 267 BP) as a function of temperature and ionic strength in three sodium phosphate buffers, pH 7.0. Persistence lengths (P) were calculated from the decay times using the theory of Hagerman and Zimm (Biopolymers 20 1481, 1981.) From the temperature dependence of P, between 4 and 25 C, the enthalpy and entropy for bending the DNA helix were obtained. For our two lowest Na^+ concentrations, 0.2 and 1 mM, the values of ΔH_B and ΔS_B were approximately zero and 0.7 to 1.4 Kcal Å/mole rad^2 K, respectively. However, the enthalpy and entropy values in 5 mM Na^+ were about 1.4 x 10^2 Kcal Å/mole rad^2 and zero, respectively. When we used the values of ΔH_B and ΔS_B determined at 5 mM Na^+ to calculate the free energy of bending needed to package T4 DNA into the phage head, we obtained ΔG_B = 8.3 x 10^2 Kcal/mole of virus. This result is less than the ΔG_B value of 1.5 x 10^3 Kcal/mole, calculated by Riemer and Bloomfield (Biopolymers 17 785, 1978) using a helix diameter of 25 Å and a value of P of 650 Å.

From the ionic strength dependence of P we have determined that the steric component of P is about 400 Å. We have found that a linear relationship exists between P_{el}, the electrostatic component of P, and the logarithm of the ionic strength. A different linear relationship was obtained for each of the fragments. At the two lowest Na^+ concentrations, we observed an appreciable increase in effective P with increasing length. This result was qualitatively consistent with the electrostatic theory of Hagerman (Biopolymers 22 811, 1983). The explanation for this phenomena is that closer contact of the ends, which results from increasing the size of the DNA molecule, leads to increased repulsion and an increase in P_{el}.

W-Pos226 EFFECT OF DISCRETE DISTRIBUTION OF IONS ON DNA - A THEORETICAL INVESTIGATION.
S. Devarajan, N. Pattabiraman and R.H. Shafer, Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143.

Using an iterative approach, monovalent and divalent ions are placed around a 20 basepair long (dC-dG) sequence in standard B and ZI conformations until the negatively charged environment around the DNA is completely neutralized. The molecule with its attendant ions in the various conformations is subjected to energy minimization using AMBER. When bound with ions, the Z form is more stable than the B form. Groove-binding provides some insight into the facility with which the B to Z transition occurs with higher charged cations. In the presence of the +2 ions, the Z form binds to more charges at the groove through more ligands, compared to the B form. The orientation around the CpG phosphates in the minor groove of the Z form is found ideal for ion-binding. For these ion-binding sites we have been able to develop molecular models. Phosphates dominate the ion-binding. Large perturbations are seen in the angles that control the phosphate orientation when an ion interacts with at least two adjacent phosphates. The binding that causes the perturbation in torsion angles is more complex in the Z form with respect to the B form. The role of the C5 methyl group in favoring the Z over the B form is analyzed by repeating the calculations after fixing the methyl groups. However, no significant effect is observed. Inclusion of solvent molecules in the calculations may bring out the role of the methyl group better. Supported by NIH Grant GM 36031.

W-Pos227 MONTE CARLO SIMULATIONS OF DNA-SALT SOLUTIONS.
M. Paulsen and M.T. Record, Jr., Departments of Chemistry and Biochemistry, University of Wisconsin, Madison, WI. 53706.

Monte Carlo simulations using a grand canonical approach were performed for a wide variety of conditions to investigate the effect of the nonideality of aqueous solutions of DNA and simple electrolytes on DNA conformational equilibria. The model and method used in this study are similar to that used previously (P. Mills, C.F. Anderson, and M.T. Record, Jr., J. Phys. Chem. 89, 3984(1985); P. Mills, C.F. Anderson, and M.T. Record, Jr., J. Phys. Chem., in press). The grand canonical approach is the approach of choice to evaluate the thermodynamic coefficients of interest including the preferential interaction parameter which is the key quantity in thermodynamic analyses of the salt-dependence of equilibria involving DNA. The behavior of the preferential interaction parameter as a function of bulk salt concentration, polymer charge density, and polymer radius was determined. The results of these simulations are compared to the predictions of more approximate statistical mechanical theories such as the Poisson-Boltzmann theory and the **counterion condensation** theory. The results of the Monte Carlo simulations are also compared to results from experimental measurements of the salt-dependence of the helix-coil transition of DNA.

W-Pos228 WATER RELEASE IN THE B-Z TRANSITION.
Donald C. Rau, LCB, National Institutes of Health, Bethesda, MD, 20892 and Holly Ho Chen, Department of Chemistry, George Mason University, Fairfax, VA 22030 (Introduced by C.T. Noguchi).

The B-Z transition of poly(dG-dC) and poly (dG-mdC) offers an opportunity to combine structure and thermodynamics to determine the energetic importance of salt and water interactions with different DNA conformations. By measuring the transition temperature for p(dGmdC) as a function of Na activity for $NaClO_4$, NaCl, and NaF solutions, we can estimate the number of water molecules released thermodynamically in the transition from the known and quantifiable effect of these anions, which span a large range of water structuring properties, on bulk solution thermodynamics. At a constant temperature, less $NaClO_4$ is necessary to effect the transition than NaCl. The ClO_4 anion, for example, is an effective water structure breaker relative to Cl^- and would be expected to increase the etropy gain from bound water release. The dependence of the transition temperature on the Na^+ ion activity relates the entropy (or enthalpy) of the transition with the number of extra Na^+ ions bound by the Z form. By comparing these curves for the different anions, we estimate that about 15 water molecules are released for every extra Na^+ concentration breadth of the transition curve at constant temperature. We find then that ΔH=+ 150 cal/mole bp, $\Delta n(Na^+)$= + .15 mole/mole bp, and $\Delta n(H_2O)$ = -2 moles/mole bp. H.H.C. is supported by NSF Grant No. DMB 850611)

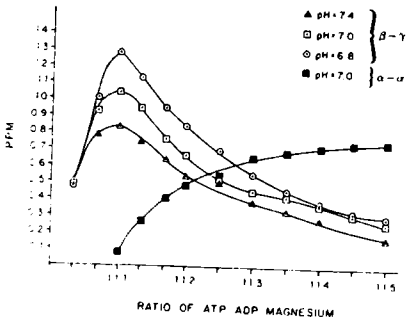
W-Pos229 CONDUCTIVITY CHANGES IN DNA SOLUTIONS DURING MELTING.

Emory H. Braswell and Arthur DeGraff III. Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268.

Manning's theory of polyelectrolyte charge condensation predicts that the effective charge on a DNA molecule in the B double helix conformation in solution will increase almost threefold upon melting. Other factors being equal, this should result in an increase in the conductivity of the solution. Although in the past conductivity measurements on DNA solutions have been performed, they have not been used significantly for following the process of melting. Using salmon testes DNA, we obtained reasonable results if the ratio of the molar concentrations of salt to that of DNA was less than about 5. Subtraction of the conductivity of the salt solution at each temperature from that of the heat denatured DNA yields a conductivity profile of the DNA alone which increases with temperature in a manner similar to, and with an equivalent conductance of about 1/3 that of the salt. A similar profile of native DNA shows an inflection at a temperature corresponding approximately to that observed in absorbance studies of the melting. Above this temperature, the conductivity rises to about that of (and acquires a similar rate of temperature increase as) the heat denatured DNA. At low temperatures where the conductivity changes are reversible, the equivalent conductivity of the native DNA is about 60% that of the heat denatured material, but the rate of temperature rise is slightly lower until the melting region is entered. Therefore, upon melting, the relative (to NaCl or native DNA) equivalent conductance of DNA increases about 50%. The implications of this less than three fold change to the structural and charge density changes are discussed.

W-Pos230 DIFFERENTIAL BINDING OF Mg^{+2} TO ATP AND ADP AS MEASURED BY ^{31}P NMR. C. Tyler Burt, Laboratory of Molecular Biophysics, PO Box 12233, MD 17-05, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.

ATP and ADP have substantially different binding constants for magnesium (Mg) (73,000 vs. 4000 M^{-1})[1] The size of this difference means that if one titrates a solution of ATP and ADP with Mg, the ATP should bind most of the initial magnesium until it is essentially saturated and then ADP should be titrated. At constant pH we find this to be true as can be seen in ^{31}P NMR spectra. There is an initial move of the γ-ATP peak downfield from the β-ADP until the ATP is Mg-saturated. This is followed by a move of β-ADP back toward γ-ATP and also the move of the α-ADP downfield from α-ATP. The size of these changes can be seen in the Figure. Some of these effects are pH sensitive as is also shown in the Figure. These results imply in vivo applications that show both ATP and ADP resonances can be used to evaluate free magnesium levels with higher sensitivity than that using only changes in ATP chemical shift.[2]



1. O'Sullivan, W.J. and Smithers, G.W., Methods in Enzymology 63:294-336.
2. Burt, C.T., Cheng, H.-M., Gonzalez, G., Barnett, P. and Chylack, Jr. L., Invest. Opthamol. Vis. Sci. 26:230.

W-Pos231 THE SALT STRENGTH DEPENDANCE OF THE COUNTERION POLARISABILITY IN SODIUM HYALURONATE. Sybren Wijmenga and Elliot Charney (Intro. by Gary Felsenfeld). National Institutes of Health, NIDDK, Bethesda, MD 20892

Electric birefringence experiments have been done on sodium hyaluronate (NaHy) samples to measure the salt strength dependence of their counterion polarisability. Recently Rau and Charney calculated the polarisability of the Debye Huckel counterion layer around a charged rod. At charge densities greater than one per 7.1 Angstroms as occurs in the nucleic acids counterion condensation occurs and both the diffuse ion layer and the condensed counterions contribute to the polarizability. Since hyaluronate has a charge density below this critical level the diffuse Debye-Huckel ion layer alone should determine the polarisability of NaHy. NaHy seems therefore a good candidate to study the polarisability of the Debye-Huckel counterion layer. The electric birefringence experiments show that the Kerr constant K, which is related to the polarisability, is approximately inversely proportional to salt strength over a wide range of salt strengths in agreement with the polarisation theory of Rau and Charney. Moreover the Kerr constant does not show a strong molar mass dependence, i.e. K is approximately proportional to the molar mass.

W-Pos232 MEASUREMENT OF FORCES BETWEEN XANTHAN POLYSACCHARIDES. Donald C. Rau and V. Adrian Parsegian, LCB, National Institutes of Health, Bethesda, MD 20892 (Introduced by M. Proutly).

Hydration forces, with characteristics very similar to those already observed between phosphotidylcholine bilayers and between DNA double helices, have now between observed to act between macromolecules of a third class, charged polysaccharides. The biopolymer xanthan has a cellulose backbone with charged (carboxylate groups) trisaccharide side chains attached to alternating glucose units. Although the exact structure is unknown, xanthan appears to adopt an ordered, probably helical, conformation in solution. By the osmotic stress technique coupled with X-ray scattering, we have directly measured the repulsive force between xanthan molecules. At close approach, an exponentially growing force with a decay length of 2.5 - 3 Å is observed. This force is independent of ionic strength from 0.1 to 1 M NaCl and is strikingly similar to the forces observed between DNA helices at about the same distances. In contrast to DNA, however, there is little difference in the amplitude of the force with NaCl or $MgCl_2$ (.02 - .1M) solutions. Another difference apparent between xanthan and DNA is that the conformation of xanthan is less well defined. Discrete breaks in the pressure vs. interaxial spacing curve suggest there are two conformational transitions over the pressure range measured. Attempts to perform equivalent measurements on other polysaccharides, such as heparin and chondroitin sulfate, are in progress. These results show the essential similarity of the perturbation of water near polar surfaces and reinforce the expectation that hydration forces are a ubiquitous feature dominating the interaction of molecules approaching contact.

W-Pos233 EFFECT OF THE GT WOBBLE BASE PAIR ON Z-DNA AND B-DNA CONFORMATIONS: A RAMAN SPECTROSCOPIC STUDY OF THE SELF-COMPLEMENTARY DNA HEXAMER d(CGCGTG). J. M. Benevides[+], A. H.-J. Wang[*] and G. J. Thomas, Jr.[+], [+]Department of Chemistry, Southeastern Mass. Univ., No. Dartmouth, MA 02747 and [*]Department of Biology, M. I. T. Cambridge, MA 02139.

The self-complementary hexanucleotide, d(CGCGTG), crystallizes as a Z-DNA double helix containing two GT base pair mismatches of the "wobble" type.[1] Comparison of the Raman spectra of d(CGCGTG) crystals with results obtained previously on two other Z-DNA crystals, d(CGCGCG) and d(CGCATGCG), shows that wobble base pairs are incorporated into the Z helix with virtually no change in geometry of the backbone phosphodiester groups. This finding is in agreement with x-ray results, which show that GT wobble pairs are accommodated without a major distortion of the Z-DNA backbone. Although the Raman spectra clearly reveal the substitution of T for C by the appearance of characteristic thymidine bands, the bands of neighboring nucleoside pairs are largely unaffected by inclusion of the wobble pairs in the Z structure. Raman spectra of d(CGCGTG) in aqueous solution are very similar to spectra of other aqueous double-stranded deoxynucleoside structures and indicate that the hexamer forms a B-DNA secondary structure in solution. Raman bands characterized previously as nucleoside conformation markers[2] indicate C2′endo/anti conformers for both dG and dT residues in the mismatched B-DNA structure. (Supported by N.I.H. Grant AI18758.)

1. P. S. Ho, C. A. Frederick, G. J. Quigley, G. A. van der Marel, J. H. van Boom, A. H.-J. Wang and A. Rich (1985) EMBO J. 4, 3617-3624.
2. G. J. Thomas, Jr., J. M. Benevides and B. Prescott (1986) Biomolecular Stereodynamics 4, 227-253.

W-Pos234 THEORY OF DYNAMIC LIGHT SCATTERING FROM RIGID DOUBLE-SPIRALS. A MODEL FOR SUPERCOILED DNA. P. G. Wu, J. M. Schurr, A. S. Benight, J. Langowski, and B. S. Fujimoto, Department of Chemistry, University of Washington, Seattle, WA 98195

We consider the possibility that tertiary structure per se is a major determinant of the apparent diffusion coefficient ($D_{app}(K)$) of weakly supercoiled DNAs at large scattering vector K. This possibility was suggested by the observation of anomalously large values of $D_{app}(K)$ at $K^2 = 20 \times 10^{10}$ cm^{-2} for complexes of single-strand binding protein (ssb) from E. coli with supercoiled pBR322 DNA. We have derived exact expressions for the initial, or first cumulant, value of $D_{app}(K)$ for rigid double spirals, and other structured objects. $D_{app}(K)$ exhibits a progression of prominent peaks at large K. These correspond to minima in the static structure factor, and nearly vanish, when the diffusion coefficient $D_{\parallel}^{R}$ for azimuthal rotation around the symmetry axis vanishes. Though inaccessible by dynamic light scattering for native superhelix densities, the first peak shifts to lower K at the edge of the observable range, when sufficient superhelical turns are lost. The pattern of changes in the experimental $D_{app}(K)$ vs. K^2 curves with increasing protein suggests that the limited binding of ssb may be highly cooperative, or all-or-none, in character.

W-Pos235 CIRCULAR DICHROISM MEASUREMENTS OF C·C$^+$ BASE PAIRS IN ANTIPARALLEL OLIGOMER DUPLEXES. Eric L. Edwards, Yuan Yang, Michael H. Patrick, Robert L. Ratliff(*), and Donald M. Gray. (Intr. by H.T. Steely, Jr.). Program in Molecular Biology, The University of Texas at Dallas, Box 830688, Richardson, TX 75083-0688 and (*)Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Box 1663, Los Alamos, NM 78545.

CD measurements of the individual oligomers $d(C_5A_{10}C_5)$ and $d(C_5T_{10}C_5)$ showed no evidence for C·C$^+$ base pair formation at temperatures $\geq$ 20°C at pH 6 or $\geq$ 0°C at pH 7 (0.5 M Na^+). 1:1 mixtures of these oligomers at 20°C, pH 6, or at 0°C, pH 7, (0.5 m Na^+) showed clear evidence for concomitant C·C$^+$ and A·T base pair formation, as monitored by an increase in the CD at 285 nm and a decrease in the CD at 249 nm, respectively. This showed that C·C$^+$ base pairs can form between antiparallel strands of a DNA duplex, in support of our previous work. (D.M. Gray, T. Cui, and R.L. Ratliff (1984) Nucl. Acids Res. 12, 7565-7580). CD evidence was also obtained for C·C$^+$ base pair formation in self-complexes of $d(\overline{CCATT})_{3,4,5,6}$ and $d(AATCC)_{4,5}$ showing that as few as two adjacent C·C$^+$ base pairs were stable in a duplex that contains 20% mismatches.

This work was supported by NIH grant GM19070, by grant AT-503 from the Robert A. Welch foundation, and by the U.S. Department of Energy.

W-Pos236 BASE PAIR ORIENTATION OF POLYNUCLEOTIDES IN SOLUTION: LINEAR DICHROISM AND MOLECULAR MECHANICAL STUDIES. Stephen P. Edmondson and Gary C. Salzman. Life Sciences Division, Los Alamos National Laboratory, Mail Stop M888, Los Alamos, NM 87545.

For polynucleotides containing only two types of bases, the base inclinations and inclination axes can be determined independently for each base by flow linear dishroism (LD) spectroscopy. However, the LD is independent of the direction (or sign) of the base inclination, and thus, four different base pair orientations are consistent with the LD data. Further, the LD measurements give no information about the backbone conformation or the helical twist and rise. To resolve the ambiguity in the base pair orientation measured for DNA in solution, we have generated double-stranded DNA molecules with different backbone conformations and different helical parameters for each of the possible base pair orientations. The helicies were then analyzed by energy minimization procedures. The results of LD studies on several different polynucleotide sequences are summarized, and models of energetically favorable structures are presented that are consistent with the base orientations observed for DNA in solution by LD.

W-Pos237 STRUCTURE OF THE TUMORIGENIC GUANINE N-2 DNA ADDUCT WITH (+) ANTI BPDE FROM MINIMIZED POTENTIAL ENERGY CALCULATIONS. B. E. Hingerty, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 and S. Broyde, Biology Dept., New York University, New York, NY 10003

We have employed a rational search strategy to compute structures of the tumorigenic adduct of (+)-7β,-8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene ((+) anti BPDE) to a DNA duplex dodecamer. In previous work we had made a global search (4,000 trials) of the conformation space of the (+) anti BPDE adduct to the deoxydinucleoside monophosphate d(CpG)(1). Both carcinogen-base stacked states and base-base stacked states of low energy were found. We have now incorporated these important forms into a duplex dodecamer in the B-DNA conformation and have minimized the energy of the larger polymers. Structures and energetic assessments will be presented.

(1) B. E. Hingerty and S. Broyde, Biopol. 24, 2279 (1985)

Supported by PHS Grant #1 RO1CA28038-06, DOE Contract #DE-AC02-81ER60015, NSF Grant #DMB8416009, and by the Office of Health and Environmental Research, U.S. Dept. of Energy under Contract #DEAC05-84OR21400 with Martin Marietta Energy Systems, Inc. (BH).

W-Pos238 A PRE-MELTING TRANSITION IN POLY d(A) POLY d(T). J. E. Herrera, M. Britt and J. B. Chaires (Intr. by H. W. Detrich, III). Department of Biochemistry, The Univeristy of Mississippi Medical Center, Jackson, MS 392164505

Poly d(A)-poly d(T) in solution shows unusual cooperative binding of many intercalators. Absorbance and circular dichroic methods were used to compare the structures of poly d(A)-poly d(T) and poly $d(A-T)_2$. The temperature dependence of daunomycin binding to both polymers was studied in an attempt to understand this unusual binding behavior. Poly d(A)-poly d(T) exhibited a pre-melting transition as measured by difference absorption spectroscopy and circular dichroism. Poly $d(A-T)_2$ exhibited comparatively minor alterations in its circular dichroic spectra and difference absorption spectra prior to melting. At low concentrations of daunomycin, increasing the temperature resulted in increased amounts of drug bound to poly d(A)-poly d(T), indicating a positive enthalpy for the drug-polymer interaction. This behavior is abolished at higher concentrations of daunomycin. Poly $d(A-T)_2$, in contrast, does not show this behavior at any daunomycin concentration and the drug-polymer interaction is characterized by a negative enthalpy. Poly d(A)-poly d(T) and poly $d(A-T)_2$ in solution have different circular dichroic spectra. With increasing concentrations of bound daunomycin, both exhibited similar changes in their spectra. These data suggest that poly d(A)-poly d(T) in solution exists in an equilibrium between two conformations and that this equilibrium can be shifted either thermally or by the binding of daunomycin. Supported by Grant CA35635 (JBC) from the National Cancer Institute.

W-Pos239 **STRUCTURE AND DYNAMICS OF BULGE-CONTAINING DEOXYRIBONUCLEOTIDES BY ^{1}H NMR.** Sarah A. Woodson & Donald M. Crothers, Department of Chemistry, Yale University, New Haven, CT 06511. (Intr. by Franklin Hutchinson)

A series of bulge-containing and normal double helical synthetic deoxyribonucleotides of sequence $^{5'}$GATGGGGCAG corresponding to a frameshift mutational hotspot in the lamda C_I gene, were compared by proton magnetic resonance spectroscopy at 500 MHz. Non-selective T1 inversion-recovery experiments were used to determine exchangeable proton lifetimes and compare helix stability and dynamics of the duplexes. An extra adenosine flanking the internal G*C base pairs has a strongly localized effect on helix stability, but the destabilizing effect of an extra cytosine in a C-tract, or guanosine in a G-tract is delocalized over the entire G*C run. These data lead to the conclusion that the position of the bulge migrates along the run in the fast exchange limit on the NMR time scale. For duplexes containing an extra G, the bulged nucleotide occupies positions in the center of the G*C tract more than those at the edge. Rapid migration of the bulge defect in homopolymeric sequences may help rationalize both frameshift mutagenesis and translational frameshifting. Non-exchangeable protons of the sequence $^{5'}$GATGGGCAG*CTGACCCATC were assigned via NOESY and COSY connectivities. Energy minimization with distance constraints was used to calculate a three dimensional structure in agreement with the NMR data. The helix is of the B family, with the extra adenine stacked in the helix.

W-Pos240 **ORIGIN AND EXTENT OF SEQUENCE-DIRECTED DNA BENDING,** Hyeon-Sook Koo, Janet Rice, & Donald M. Crothers, Department of Chemistry, Yale University, New Haven, CT 06511.

DNA fragments having homopolymeric adenine•thymine tracts phased with the helix screw are thought to be bent, on the basis of their various physical properties. According to our model, adenine•thymine tracts adopt a polymorphic structure (H-DNA), and bending occurs at the junctions between the A•T tract and adjacent B-DNA. To find the features in A•T base pairs that drive A tracts into the H-form, different base analogues such as uridine, 5-methyl cytidine,and inosine, in addition to the 4 ordinary bases were incorporated into oligonucleotides. Ligated multimers of 10 bp long synthetic oligonucleotide duplexes were run on polyacrylamide gels. By comparison of gel mobility data for different sequences, factors both necessary and irrelevant for the formation of H-DNA were identified.

Rates of DNA ligase-mediated covalent ring closure for DNA fragments containing multiple bend loci provide a measure of the ring closure probabilities (j factors) for the sequence. The-dependence of the j factor on DNA length gives an estimate of the helix screw of the sequence; bend angles at the junctions between the A•T tract and B-DNA are determined by comparing the experimental j factors with values predicted by theoretical calculations.

W-Pos241 A TWO-DIMENSIONAL NMR STUDY OF THE SOLUTION STRUCTURE OF A ALTERNATING PYRIMIDINE-PURINE SEQUENCE. James D. Baleja and Brian D. Sykes. MRC Group in Protein Structure and Function and the Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7; and Johan H. van de Sande. Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

High resolution NMR techniques have been used to examine the structural and dynamic features of the self-complementary DNA octamer $d(CATGCATG)_2$. Proton resonances have been assigned by using two dimensional techniques, demonstrating through bond and through space connectivities from COSY and NOESY spectra, respectively. Directional effects in the duplex are indicated by base proton chemical shifts with base protons nearer the 5' end generally resonating at lower field relative to protons of the same base type nearer the 3' end. Furthermore, this effect is seen to decrease when comparing bases closer to the 3' end. A large number of short (<5Å) approximate interproton distances using nuclear Overhauser enhancement (n.O.e.) measurements have been obtained by volume integration of n.O.e. cross-peaks in NOESY spectra. The distance data are consistent with an overall B-type conformation but with some degree of local structural variation.

W-Pos242 SINGLE BASE BULGES IN SMALL RNA HAIRPINS ENHANCE ETHIDIUM BINDING. S. A. White and D. E. Draper, Chemistry Department, The Johns Hopkins University, Baltimore, MD 21218.

A series of four RNA hairpins containing a single base loop has been synthesized *in vitro* using T7 polymerase. In previous studies of ribosomal RNA, an unusually strong ethidium binding site was discovered in a helix having a bulged A residue. [J. M. Kean, S. A. White and D. E. Draper, Biochemistry, *24*, 5062 (1985)]. Thus, small hairpins were designed to explore the features necessary for a strong drug binding site. While maintaining the same base pair sequence around the site, the bulge was varied from A to U or C or deleted. Both methidiumpropylEDTA(Fe(II)) and ethidium bind preferentially to the lone CpG sequence whether or not the bulge is present. Each of the hairpins having a bulged base binds ethidium 10 fold more strongly than the bulgeless helix. Cleavage experiments with cobra venom ribonuclease indicate that major phosphate backbone conformational changes occur up to several base pairs away from the binding site as ethidium binds to the bulge-containing helices. These results indicate that the bulged base itself may be less important than the additional phosphodiester linkage which could introduce conformational flexibility. Such increased helical backbone flexibility may play an important role in the enhanced intercalative binding seen in the bulged helices. The role of bulge position, sequence effects, and a possible 'allosteric' switch will also be discussed.

W-Pos243 USE OF SITE-DIRECTED MUTAGENESIS IN THE ASSIGNMENT AND INTERPRETATION OF 5S RNA PROTON NMR SPECTRA. Daniel T. Gewirth and Peter B. Moore, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511.

The complete assignment and interpretation of the downfield proton nmr spectrum of E. coli rrnB 5S RNA is hindered by severe spectral overlaps and incomplete NOE connectivities between adjacent basepairs. One possibility for overcoming these limitations is to use site-directed mutagenesis to specifically alter individual basepairs in 5S RNA. By correlating the changes in the nmr spectrum of the mutated RNA with the sequence changes, it may be possible to make firm assignments of the wild-type molecule. Site-directed mutagenesis can certainly be used to 'design' the downfield proton nmr spectrum of 5S RNA to simplify the spectrum and create readily observable 'reporter' resonances. These effects have helped the study of the 5S RNA - L18 protein complex. Finally, site-directed mutagenesis has been used to delete large regions of the wild-type 5S RNA to create smaller domains suitable for detailed physical study.

W-Pos244 **THE WAVELENGTH DEPENDENCE FOR THE PHOTOCROSSLINKING OF DNA-PSORALEN MONOADDUCTS**

Yun-bo Shi and John E. Hearst, Dept. of Chemistry, University of California, Berkeley, CA 94720.

The photoreactions of HMT (4′-hydroxymethyl-4, 5′, 8-trimethylpsoralen) monoadducts in double-stranded DNA have been studied using complementary oligonucleotides. The HMT was first attached to the thymidine residue in the oligonucleotide 5′-GAAGCTACGAGC-3′ as either a furan-side monoadduct or a pyrone-side monoadduct. The HMT monoadducted oligonucleotide was then hybridized to the complementary oligonucleotide 5′-GCTCGTAGCTTC-3′ and irradiated with monochromatic light. In the case of the pyrone-side monoadducted oligonucleotide photoreversal was the predominant reaction and very little crosslink was formed at all wavelengths. The course of the photoreaction of the double-stranded furan-side monoadducted oligonucleotide was dependent on the irradiation wavelength. At wavelengths below 313 nm both photoreversal and photocrosslinking occurred. At wavelengths above 313 nm photoreversal of the monoadduct could not be detected and photocrosslinking occurred efficiently with a quantum yield of 2.4×10^{-2}. (This work has been supported by NIH Grant #GM 11180.)

W-Pos245 CD OF TWO CONFORMATIONS OF POLY[d(G-C)] INDUCED BY LOW pH. Vincent P. Antao, Carla W. Gray, Robert L. Ratliff(*), and Donald M. Gray. Program in Molecular Biology, The University of Texas at Dallas, Box 830688, Richardson, TX 75083-0688, and (*)Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Box 1663, Los Alamos, NM 78545.

CD and absorption spectra showed that poly[d(G-C)] (at 0.09M NaCl, 0.01M Na^+(phosphate), 20°C) underwent two conformational transitions as the pH was lowered by the addition of HCl. The first transition was complete at about pH 3.0. The second transition was complete upon lowering the pH to 2.6. The second transition could also be induced at pH 3.0 by heating the polymer to 40°C or by allowing the sample to stand at room temperature for several hours. The CD spectrum for the second acid conformation had large CD bands including a positive one at 288nm, a characteristic associated with $C \cdot C^+$ base-pairs. Proton uptake experiments showed that 0.25 proton per base, or one proton per two cytosines, was taken up by the polymer at pH 3.0. No additional proton-uptake was required to induce the second transition. Electron microscopy showed that the DNA in both acid conformations had a diameter of about 2-3nm and that there was no significant condensation into supramolecular aggregates for either acid form. A possible interpretation of our data is that the first form of poly[d(G-C)] consisted of about 50% protonated $G \cdot C^+$ base-pairs while the second form consisted of $C \cdot C^+$ base-pairs alternating with G•G base-pairs.

This work was supported by NIH grants GM19070 and GM34290, by Grant AT-503 from the Robert A. Welch Foundation, and by the U.S. Department of Energy.

W-Pos246 GEOMETRY OF A STABLE DNA 4-WAY JUNCTION. Julia P. Cooper and Paul J. Hagerman, Department of Biochemistry, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

General genetic recombination is thought to be initiated by a pair of single strand exchanges between homologous DNA duplexes, generating a branched intermediate called the Holliday structure. 4-way DNA junctions (or cruciform junctions) provide useful experimental models for the Holliday intermediate. These structures are recognized specifically and cleaved by resolving enzymes, such as T4 endonuclease VII, which display little sequence specificity. This observation suggests that resolving enzymes may recognize DNA junctions on the basis of their geometry. In order to explore this possibility, we have constructed a stable (60-base pair) 4-way junction using synthetic DNA, and are currently determining its geometry. Our experimental approach utilizes the fact that bent DNA molecules demonstrate reduced mobilities on polyacrylamide gels to an extent that varies with the degree of the bend angle. From the 60-base pair 4-way junction, we have produced a set of molecules in which all pairwise combinations of junction arms have been extended by 98 base pairs (continuous phosphodiester backbones). By comparing the electrophoretic mobilities of these molecules on polyacrylamide gels, we can deduce qualitatively the relative angles between each pair of elongated junction arms. We have observed that the pairwise combinations have widely differing electrophoretic mobilities, indicating that the junction is not a completely flexible structure, nor is it tetrahedral or a right planar cross. Studies of this type will help elucidate the factors that control interactions between resolving enzymes and DNA.

W-Pos247 EVIDENCE FOR Z-FORM IN POLY d(A-C)•POLY d(G-T) AND OTHER POLYDEOXYNUCLEOTIDES BY VACUUM UV CIRCULAR DICHROISM. Jeannine H. Riazance and W. Curtis Johnson, Jr., Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA, and Lawrence P. McIntosh and Thomas M. Jovin, Max-Planck-Institut für biophysikalische Chemie, Abtelung Molekulare Biologie, Postfach 968, D-3400 Göttingen, FRG.

Circular dichroism spectra are extended into the vacuum UV to about 178 nm for four polydeoxynucleotides of various sequences capable of assuming the left-handed Z form. It is found that each of these polymers, including those with the four different bases, have a characteristic negative feature at short wavelengths when in the Z form. In contrast, the B form only has a positive band between 180 and 200 nm. Furthermore, a blue shift of the short wavelength crossover appears independent of sequence and thus universally diagnostic of the B- to Z-form transition. Our results confirm that poly d(A-C)•poly d(G-T) can assume the Z form in dilute solution.

W-Pos248 CIRCULAR DICHROISM STUDIES OF THE BINDING OF ETHIDIUM BROMIDE TO DNA RESTRICTION FRAGMENTS. Elizabeth Slobodyansky and Nancy C. Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

The equilibrium binding of ethidium bromide (EB) to DNA restriction fragments (2936 bp, 1426 bp and two of 160 bp with different sequences, 12A and 12B) was investigated using circular dichroism (CD). CD spectra of the DNA-EB complexes have been measured as a function of added dye. Difference spectra have been calculated by subtracting the spectrum of the pure DNA from the spectra of DNA-EB complexes of different dye/phosphate (D/P) ratios. The difference CD spectra are characterized by four major bands with extrema at 310, 275, 207, and 193 nm. The band at 310 nm is the most sensitive to the size and sequence of the DNA. The dependence of $\Delta\varepsilon_{310}$ on D/P for the 2936 bp fragment is about the same as for calf thymus DNA, reaching a plateau at D/P of 0.2-0.3 and remaining constant until D/P = 1.0. The amplitude of $\Delta\varepsilon_{310}$ is lower for the 1436 bp fragment, and the behavior of fragments 12A and 12B are different from each other and from the 2936 bp fragment in this region. The bands at 275 and 207 nm look similar for all restriction fragments. Both bands were found to have midpoints at D/P$\sim$0.5, corresponding to one molecule of dye bound per base pair. The band at 193 nm is positive and has approximately the same value for calf thymus DNA and the 2936 bp, 1426 bp, and 12B fragments. The magnitude is smaller for fragment 12A. This band decreases in amplitude upon intercalation of EB, reaching negative values at D/P$\sim$0.1. The decrease in amplitude may reflect unwinding of the DNA upon intercalation of the dye.

W-Pos249 TOWARDS A BALANCED PHYSICAL PICTURE OF DNA STATICS AND DYNAMICS:

M. Hogan (Dept. of Molecular Biology) and R.H. Austin (Dept. of Physics), Princeton University, Princeton, NJ 08544

Despite a recent explosion of interest in "bent" DNA, little effort has been made using relevant and unambiguous physical techniques to verify that the DNA sequences are indeed bent statically. Many of the researchers have blithely forgotten that DNA is indeed a molecule with a quite finite Young's modulus and twisting modulus and have been trapped by the seductive static images that x-ray diffraction provides, thereby ignoring any dynamical considerations.

In this poster we will outline the physical evidence for static bends vs. dynamical bending, primarily using triplet anisotropy decay, and try to interpret our own gel work and others to determine the extent to which DNA is truly statically bent vs. thermal induced bending. We will include our own work on the base-pair sequence dependence of DNA flexibility. If we are wrong and all the effects observed on gels are indeed due to static bends, we will admit it. Tune in for details.

W-Pos250 **DNA CONFORMATION DURING FIELD-INVERSION GEL ELECTROPHORESIS (FIGE)**

Daniel P. Moore, John A. Schellman and Walter A. Baase
Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229

Large double-stranded DNAs, on the order of 1,000 kilobasepairs (kbp), have recently been electrophoretically resolved in 1% agarose gels by a technique which periodically reverses the electric field [G.F. Carle, M. Frank and M.V. Olson, Science 232, 65-68, (1986)]. Although the mobility of long DNA in agarose is approximately independent of the molecular weight in an unswitched electric field, field-inversion gel electrophoresis can be used to separate such long molecules because a given length of DNA has a range of switching intervals which result in reduced mobility.

We have made time dependent linear dichroism measurements at 253.7 nm to directly determine the degree of orientation of double-stranded DNA during field-inversion gel electrophoresis for T7 DNA (40 kbp), T4 DNA (180 kbp) and G DNA (750 kbp). We find a 50% (for T7) to 75% (for G) transient decrease of the steady state dichroism upon field reversal. This loss of dichroism occurs faster than simple decay of dichroism upon field removal. Furthermore, the period of reorientation seen by means of linear dichroism corresponds to the forward pulse times which give reduced mobility. In addition, the orientation of each DNA goes through a transient maximum just prior to reaching a steady state value. Finally, for a given DNA, the orientation and reorientation kinetics as well as the magnitude of the steady state dichroism are primarily functions of the electric field strength. This behavior is characteristic of a damped oscillator and lends experimental support to the idea of a transient low mobility conformation. It also suggests refinements to the reptation model for DNA migration in agarose gels. (Supported by PHS Grant GM20195, NSF Grant DMB 8609113 and a Summer Fellowship from the American Heart Association to DPM.)

W-Pos251 STUDY OF THE SEQUENCE-DEPENDENT STRUCTURE IN B-DNA DOUBLE HELICES WITH METROPOLIS ALGORITHM. Chang-Shung Tung, T-10, MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545.

In this study, basepair geometries (helix twist angle, propeller twist angle, change of roll angle, basepair separation, etc.) are treated the same way as in the previous model (J. Biol. Chem. 261, 3700-3709, 1986). In contrast to the previous model, sugar-phosphate atoms are included explicitly. These backbone atoms are only allowed to move with a few degrees of freedom that include glycosyl angle, pseudorotational angle, and a rotation for the phosphate group with respect to a pseudobond connecting between O3' atom of one sugar and O5' of the next sugar.

With this reduced set of coordinates, statistically averaged conformation of DNA double helices were calculated with Metropolis Algorithm (Monte Carlo Method). All possible tetramer (four basepairs) sequences were studied. The results can be used for predicting the sequence-dependent structure in B-DNA double helices.

W-Pos252 ADENINE-THYMINE TRACTS ADOPT AN UNUSUAL CONFORMATION IN KINETOPLAST DNA, Amanda Milgram Burkhoff* and Thomas D. Tullius *+ (Intro. by Saul Roseman), *Department of Biology and +Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218.

Kinetoplast DNA has been shown to be stably curved. This phenomenon has been attributed to the presence of periodically spaced runs of Adenines (A tracts), and has been referred to as sequence-directed bending. Different mechanisms have been proposed to explain how such poly(A) sequences could be responsible for DNA bending. One model attributes bending to wedges at ApA dinucleotides. Another model hypothesizes that bends result at the junction of A tracts and B-DNA. To test these models, we studied the hydroxyl radical cutting pattern of a fragment of a mini-circle from the trypanosome Crithidia fasciculata. Hydroxyl radicals were generated via reaction of hydrogen peroxide with iron(II) EDTA. Such cutting patterns have been shown to reveal subtle structural variations in the DNA helix. With most DNA molecules every position along the backbone is cut nearly equally by hydroxyl radical. We show that the cutting pattern for kinetoplast DNA, in contrast, is strikingly sinusoidal, with the positions of the runs of adenines in sharp register with the sine wave. At the adenine tracts, reactivity of the backbone toward hydroxyl radical was progressively reduced in the 5' → 3' direction, revealing cooperative structural changes adopted by these sequences. We also show that a single tract of 5 Adenines in PBR 322 has the same structural characteristics as the kinetoplast A tracts. Therefore, neither the wedge nor junction model is completely consistent with our results. Any model, explaining the reactivity pattern of the A tracts must incorporate both the smooth, progressive change in structure and the different affects on the backbone at the 5' and 3' side of the A tract.

W-Pos253 WATER CHANNELS ARE PRESENT IN RENAL PROXIMAL TUBULE CELL MEMBRANES. Mary M. Meyer and A.S. Verkman. Cardiovascular Research Inst., Univ. of Calif., San Francisco, CA 94143.

Water transport mechanisms in rabbit proximal convoluted tubule (PCT) cell membranes were examined by measurement of: (1) osmotic [P_f] and diffusional [P_d] water permeability coefficients, (2) inhibition of water transport by mercurials, and (3) activation energies [E_a] for water transport. P_f was measured in PCT brush border (BBMV) and basolateral membrane vesicles (BLMV) and in viable PCT cells by stopped-flow light scattering. P_d was measured in intact PCTs and in PCT cells by proton NMR T_1 relaxation times using extracellular Mn as a paramagnetic quencher. In BBMV, P_f (0.01 cm/s, 23°C) was inhibited 63% by 300 μM Hg^{++} (K_I = 138 μM); E_a increased from 2.7 to 10.1 kcal/mole (15-35°C) with 300 μM Hg^{++}. In BLMV, P_f (0.018 cm/s, 23°C) was inhibited 72% by Hg^{++} (K_I = 63 μM); E_a increased from 4.2 to 7.3 kcal/mole with 300 μM Hg^{++}. Mercurial inhibition was reversed with 10 mM mercaptoethanol. Basolateral membrane P_d determined by NMR in suspended PCTs was 2 x 10^{-3} cm/s, with E_a 2.9 kcal/mole and 60% inhibition by 5 mM pCMBS. In PCT cells which resemble the brush border membrane, P_f/P_d = 3.2 with E_a = 13 kcal/mole (>35°C) and 6 kcal/mole (<35°C). Cell P_f was inhibited 60% by 300 μM Hg^{++} and independent of osmotic gradient size, flow direction and initial cell volume, indicating absence of unstirred layer or flow-dependent effects. The rapid transport rates, reversible inhibition by mercurials, low activation energies which increase with mercurials and P_f/P_d >1 provide strong evidence for the presence of water channels in PCT brush border and basolateral membranes. These channels are similar to those found in erythrocytes and are likely required for rapid PCT transcellular osmotic water transport.

W-Pos254 ABNORMALITIES IN SURFACE MEMBRANE LIPID MOBILITY IN MALIGNANT HYPERTHERMIA. J.R. Mickelson, J.A. Ross, H.S. Thatte, S.M. Lewis, D.D. Thomas and C.F. Louis. Dept. of Veterinary Biology, University of Minnesota, St. Paul, Minn. 55108.

Malignant hyperthermia (MH) is an inherited metabolic disorder, generally believed to result from a defect in skeletal muscle calcium regulation. Alterations in calcium transport by membranes from both muscle and non-muscle tissues have been reported, however, suggestive of a generalized membrane defect in MH. To investigate the possibility of altered plasma membrane function in MH, the rotational dynamics of lipid motion in erythrocyte and skeletal muscle sarcolemma (SL) membranes from MH and normal pigs was examined by EPR spectroscopy. In both erythrocyte ghost membranes and intact erythrocytes, at all temp. from 2° - 40°C, the rotational motion of the spin label 16-doxyl stearic acid (16-NS) was greater in normal than in MH membranes. There were no breaks in the Arrhenius plots of probe motion in the erythrocyte ghosts; however, the apparent activation energy (Ea) for probe motion was significantly different in these two membranes (Ea = 2.83 ± 0.20 and 2.43 ± 0.16 kcal/mole, for normal and MH respectively). While there were no breaks in the Arrhenius plots of 16-NS motion in normal intact erythrocytes (Ea = 2.81 ± 0.36 kcal/mole), there were breaks in the plots of probe motion at both 24° and 33°C in intact MH erythrocytes (Ea = 3.61 ± 0.36 kcal/mole below 24°, and 1.84 ± 0.45 kcal/mole above 24°C). The Arrhenius plots of 16-NS motion in isolated SL membranes also showed significantly less probe motion in MH than in normal SL over the range 2° - 40°C, although the Ea were similar (Ea = 3.33 kcal/mole). Based on studies demonstrating altered lipid mobility in membranes (both SL and SR) from skeletal muscle, and also from erythrocytes, we conclude that there may be a generalized membrane defect in MH. (Supported by NIH GM-31382).

W-Pos255 ELECTRON MICROSCOPY OF THE INTACT SKELETON OF STRETCHED HUMAN ERYTHROCYTE MEMBRANE. F. Jung and F. Sachs, Dept. of Biophysics, State University of New York, Buffalo, NY 14214

In our study of stretch-activated ion channels, one goal is to develop a technique for visualization and immunolocalization of the cytoskeleton components, particularly those of the spectrin network, at the ultrastructural level. We have applied a modified version of such a technique developed by Byers and Branton (Proc. Natl. Acad. Sci. (USA), 82:6153-6157 (1985)) which involves artificially spreading out the spectrin network of human erythrocytes. Our modified technique uses thicker support films (0.4% Formvar) cast onto glass slides. The thicker film provides sufficient support to allowing us to eliminate the mounting of grids on Vaseline and cleaning with benzene (T. Byers, personal communication). Our results confirm the major findings of Branton's and Steck's (J. Cell Biol., 102:997-1006 (1986)) groups: (1) The spectrin network is a regular lattice of polygons; (2) Globular structures (1 to 2), presumably ankyrin-band 3, attach to the middle of each spectrin; (3) Spectrin can be stretched mechanically to about 200 nm from an initial length of about 60 nm in an unperturbed membrane. A new finding is the existance of sharp gradients of tension on the membrane during sample preparation, so that expanded regions occur immediately (within about 200 nm) adjacent to condensed regions. This separation may reflect regions where the membrane separates from the support film.

Supported by NIADDK DK37792-09A1 and USARO 22560-LS.

W-Pos256 THE BAND-8 PROTEIN AND METHEMOGLOBIN REDUCTASE MECHANISM, N. Thieda, H. Mizukami, D. E. Bartnicki, and Y. Lyles. Division of Regulatory Biology and Biophysics, Department of Biological Sciences, Wayne State University, Detroit, Michigan. 48202

The structure and function of the band-8 protein of human erythrocytes have remained obscure. We now report that the protein interacts with several cytoplasmic and membranous proteins under the influence of calcium. It is now evident that it binds methemoglobin, but not oxyhemoglobin.

Washed human erythrocytes were hemolyzed and the band-8 protein was extracted in 0.1 M NaCl at pH 7.8. The protein was purified chromatographically, and its purity was confirmed with SDS-PAGE. The protein was made of two pairs of acidic 24,000 dalton subunits. Use of a band-8 labeled affinity column revealed that methemolgobin bound to the protein, but not oxyhemoglobin. Furthermore, only in the presence of calcium did significant quantities of the other erythrocyte proteins bound to the band-8 protein. Among these were spectrin, and several cytoplamic proteins. It was also discovered that a significant quantity of a pair of low molecular weight (11,000 and 13,000 daltons) proteins were bound only in the presence of calcium.

These features make us believe that the band-8 protein might be involved in the methemoglobin reductase mechanism which could be regulated by calcium. (Supported in parts by the funding from the American Heart Association of Michigan)

W-Pos257 DYNAMICS AND INTERACTIONS OF THE ANION CHANNEL IN INTACT ERYTHROCYTES AND GHOST MEMBRANES: EFFECTS OF CYTOSKELETON REMOVAL AND PROTEIN CROSS-LINKING.

A.H. Beth, T.E. Conturo, P.S.R. Anjaneyulu, and J.V. Staros, Department of Molecular Physiology and Biophysics and Department of Biochemistry, Vanderbilt University, Nashville, TN 37232

In previous work we have described the development of a new membrane-impermeant bifunctional spin-labeling reagent (bis(sulfosuccinimidyl)-4-doxyl pimelate, (BSSDP)) which has allowed us to affinity spin-label the anion channel (band 3) in intact human erythrocytes (Beth et al., (1986) Biochemistry 25, 3824-3832). This reagent forms an intramolecular cross-link which spans residues on the 38 kDa and 58 kDa peptides produced by chymotryptic digestion of the anion channel. Other work has demonstrated that reaction of BSSDP with the anion channel can be blocked by pretreatment of intact erythrocytes with the anion transport inhibitor DIDS (Beth et al., (1986) in Proceedings of the First Membrane Protein Symposium, in press) suggesting that the spin label reagent is reacting with the DIDS site or an overlapping site on the protein. We have employed saturation transfer EPR (ST-EPR) spectroscopy to show that the rotational diffusion of the anion channel is significantly enhanced in ghost membranes relative to intact cells, a result which has been interpreted as resulting from altered interactions of the anion channel with cytoskeletal proteins. Recent work has shown that neither extraction of bands 1, 2, and 5 from ghost membranes nor covalent cross-linking of monomers of the anion channel to covalent dimers in the extracellular domain with BSSS (bis(sulfosuccimidyl)suberate) strongly influences the rotational mobility of the anion channel. These latter results on ghost membranes are consistent with the optical studies of Cherry and coworkers. Our data suggest that interactions of the anion channel with membrane or cytoskeletal components other than the spectrin-actin network influence the mobility of the channel in the membrane. Supported by: NIH HL34737 and the Chicago Community Trust/Searle Scholars Program (AHB) and NIH DK31880 (JVS).

W-Pos258 RED CELL CYTOSKELETAL PROTEIN/LIPID MONOLAYER INTERACTIONS OBSERVED BY A MODIFIED WILHELMY SURFACE TENSION MEASUREMENT TECHNIQUE. By William T. Potter, Pamela J. Brandt, John A. Norman, Kingman Ng, Ellis S. Benson, and Andreas Rosenberg.

Lipid monolayers provide a well-defined, manipulative medium to study the reconstruction of red cell cytoskeletal structural elements in vitro. The measured surface tension at the vapor/liquid interface is related to the association and/or incorporation between lipid and protein components. The commonly used Wilhelmy technique involves measurements of the weight difference between a wettable thin plate partially immersed in a pure solvent versus that with a lipid and/ or protein layer at the surface. The weight of the plate depends upon the surface tension as well as a plate buoyancy correction factor. Typical Wilhelmy measurements use a single plate and attempt to avoid the buoyancy correction term by having negligible immersion. This methodology, however, results in poor experimental reproducibility and sensitivity from minor variations in either the liquid/ plate contact wetting angle or variations of less than 1 mm in the depth of plate immersion. Increases in both the sensitivity and the ease of experimental methodology are presented here by using a double-beam balance system with two interconnected reservoirs maintained at constant fluid level height. Minor variations in submersion levels of the two plate system are initially zeroed and the weight change upon formation of a lipid/protein layer at the interface are measured directly with a constant displacement electrobalance. Surface pressure versus area measurements support distinct changes in the association of spectrin and other components of the red cell cytoskeletal network to the monolayer upon variations in phospholipid, cations and pH.

W-Pos259 BIOPHYSICAL ANALYSIS OF NOVEL TRANSPORT PATHWAYS INDUCED IN RED BLOOD CELL MEMBRANES.
Hagai Ginsburg and Wilfred D. Stein, Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel.

Human erythrocytes pulsed with high transmembrane voltages, or oxidized in the presence of diamide, or treated with toxins, or subjected to infection by malarial parasites, reveal new transport pathways which allow transmembrane movements of ions and non-electrolytes. We have analyzed the data (collected by Deuticke and his colleagues: Biochim. Biophys. Acta, 731 (1983) 196; 816 (1985) 332; 820 (1985) 173) and by ourselves (Mol. Biochem. Parasitol., 8 (1983) 177; 14 (1985) 313) in terms of models for these pathways. A pore model, fitting the Renkin equation, fits the toxin data accurately. The path opened by oxidation behaves as a non-Stokesian (Lieb and Stein, J. Memb. Biol. (1986)) barrier, with hydrophilic characteristics. We suggest that this path is formed between hydrophilic side chains of membrane-straddling proteins. The malaria-induced path seems to be through a very loosely-structured, weakly hydrophobic region which we consider to be formed between the phospholipid head-groups and the membrane proteins. The electrically-induced pathway could not be unambiguously characterized by our analysis.

W-Pos260 EFFECT OF DEOXYGENATION RATE ON FORMATION OF IRREVERSIBLY SICKLED CELLS
Kazumi Horiuchi and Toshio Asakura, The Children's Hospital of Philadelphia, Dept of Pediatrics and Dept of Biochemistry and Biophysics, Univ. of Pennsylvania, Philadelphia, PA19104

The effect of deoxygenation rate on the formation of irreversibly sickled cells (ISCs) were investigated using metabolically replete SS cells. We found that the formation of ISCs required Ca^{2+} and that the rate of the formation depended on the rate of deoxygenation. When less dense SS cells were slowly deoxygenated by flushing with nitrogen gas containing 5% CO_2 at a rate of 3 ml/min, the content of ISCs increased from 5% to 26.5% after 24 hr. In contrst, ISC formation was reduced significantly when SS cells were deoxygenated rapidly (10,35 ml/min). More number of typical sickle-shape cells were formed upon slow deoxygenation than upon quick deoxygenation. The formation of ISCs appeared to be related to the ion transport through the cell membrane. The potassium content decreased more significantly during slow deoxygenation than that by rapid deoxygenations. Since the sodium increase of influx was less than that of potassium efflux, the density of SS cells increased more strongly for cells deoxygenated slowly. To express the membrane deformation of deoxygenated cells numerically, we chose "maximum cell length (MCL)" as a first approximation. The mean MCL of slowly deoxygenated cells after 24 hr of incubation was about 2.5 times greater (20.5 ± 7.0 um) than initial mean MCL (8.2 ± 1.0 um). In contrast, the mean MCL for quick deoxygenation was only 12.3 ± 5.0 um. The relationship between ISC formation and MCLs was found to be linearly correlated (cor. coef. = 0.97). From these results, we concluded that the degree of the formation of ISCs depended on the length of sickled cells formed under deoxygenated condition, suggesting that membrane stretching is an important factor for the cell dehydration and irreversible deformation of membranes. (supported by NIH grant HL20750)

W-Pos261 MECHANISM OF INHIBITION OF ANION EXCHANGE IN HUMAN ERYTHROCYTES BY A MEMBRANE-IMPERMEANT CARBODIIMIDE.
[1]P.K. Werner and [2]R.A.F. Reithmeier, [1]Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7 and [2]Membrane Biology Group, Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

The importance of aspartate or glutamate residues in the anion exchange function of the human erythrocyte Band 3 protein has been suggested by the pH dependence of transport, and could be confirmed by inhibition of anion exchange by a carboxyl-directed reagent, 1-ethyl-3-(3-trimethylaminopropyl) carbodiimide (ETC). Treatment of intact human erythrocytes with ETC in a citrate-buffered sucrose medium resulted in irreversible inhibition of phosphate-chloride exchange and the level of inhibition achieved was dependent on the concentration of citrate present during treatment. Under the conditions that led to inhibition, [^{14}C]-citrate was incorporated into Band 3 with a stoichiometry of 2.1 nmol citrate per mg ghost protein. Fragmentation of Band 3 indicated about 90% of this rabiolabel was in the 60 kDa chymotryptic fragment and, after further cleavage, remained associated with the 17 kDa chymotryptic fragment. This modification of Band 3 was accompanied by a loss of [^{3}H]H_2DIDS covalent labeling, yet noncovalent binding of stilbenedisulfonates was retained. Our interpretation is that the inhibition in citrate-containing media is due to ETC-activated citrate modification of lysine *a*, the residue that reacts covalently with [^{3}H]H_2DIDS, resulting in altered transport function. Treatment with ETC in the absence of citrate resulted in inhibition of anion exchange that was reversed upon prolonged incubation. This reversal was prevented by addition of certain exogenous nucleophiles. Thus, inhibition of anion exchange by ETC in citrate-free media appears to involve modification of a protein carboxyl residue. (Supported by the Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada.)

W-Pos262 MEMBRANE CHLORIDE TRANSPORT MEASURED USING A CHLORIDE-SENSITIVE FLUORESCENT PROBE. Nicholas P. Illsley and A.S. Verkman. CVRI, UCSF, San Francisco, CA 94143.

A new method for the measurement of membrane Cl transport has been developed which is sensitive to changes in [Cl] of 1 mM, has a rapid response time (<1 ms), and can be used for electrogenic and electroneutral transport measurements. It is based on the quenching of a fluorescent dye (6-methoxy-[3-sulfopropyl]quinolinium; SPQ) by Cl. Cl quenches SPQ fluorescence (ex. 350 nm; em. >420 nm) by a collisional mechanism (K_q=118 M^{-1}) as determined by heterogeneity analysis of phase and modulation lifetimes. SPQ fluorescence was also quenched by Br, I and SCN (K_q=197, 282, and 225 M^{-1}), but not by HCO_3, PO_4, SO_4, Na, K, Ca, ionic strength and pH (5-9). Excitation and emission spectra were unaltered by the addition of quenchers. To validate the method in an established system, erythrocyte ghosts were loaded with 10 mM SPQ for 15 min at 37°C and extracellular dye was washed off. The Cl efflux rate in ghosts containing 100 mM Cl were mixed with isosmotic SO_4 at 37°C was 0.600 ± 0.008 μmol/s·mg membrane protein (mean ± s.d.; n=3) which decreased to 0.021 ± 0.013 μmol/s·mg in the presence of the stilbene inhibitor, H_2DIDS (0.1 mM). Addition of ghosts to a buffer containing 25 mM HCO_3 gave a rate of 3.33 μmol/s·mg, decreased to 0.05 μmol/s·mg in the presence of 0.5 mM H_2DIDS. Experiments measuring $^{35}SO_4$ uptake rates and H_2DIDS inhibition confirmed that changes in SPQ fluorescence reflect Cl/anion exchange activity accurately and that SPQ does not itself alter transport rates. The studies presented here show that measurement of SPQ fluorescence is a simple yet powerful method for studying Cl transport without restrictions of other methods including ^{36}Cl uptake, patch-clamp and microelectrodes. It is likely that Cl transport measurement using SPQ will find wide applications in vesicles, cells and intact tissues.

W-Pos263 IS THE RED CELL UREA TRANSPORTER A CARRIER OR A CHANNEL? O. Fröhlich and D. Trammell. Department of Physiology, Emory University School of Medicine, Atlanta GA 30322.

When tracer urea efflux was measured from erythrocytes, its rate decreased when the concentration of (cold) extracellular urea was increased. This is not typical for a carrier kinetic mechanism where normally trans-acceleration is observed, but it is not inconsistent with an asymmetric carrier mechanism. We studied this phenomenon in more detail with the more slowly transported thiourea (TU) which permitted us to measure tracer efflux and influx. Using the inhibitor thionicotinamide at different concentrations and extrapolating to saturating concentrations, we dissected the fluxes into mediated transport and passive diffusion. At 0°C and 70 mM, the passive diffusive flux was 22 mmol (kg solids)$^{-1}$ s^{-1}, corresponding to a permeability of 1.2 x 10^{-7} cm s^{-1}. Under the same conditions, mediated tracer TU_i efflux decreased hyperbolically from 95 mmol (kg solids)$^{-1}$ s^{-1} (at TU_o=0) to 28 mmol (kg solids)$^{-1}$ s^{-1} (at TU_o=70 mM), with an apparent inhibitor constant of 4 mM. This trans-inhibition of 70% excludes a symmetrical carrier (with the same translocation rates in both directions). In order to test whether a strongly asymmetric carrier mechanism is still possible, we measured the rates of tracer TU influx into cells containing 0-70 mM TU. In these experiments there was no significant dependence of tracer influx on TU_i. In order to exclude a carrier mechanism, one would have needed a trans-inhibition of at least 20%. In terms of these experiments, therefore, we cannot exclude a strongly asymmetric carrier kinetic mechanism. However, we would also have to assume that the translocation reaction of the unloaded hypothetical carrier is significantly higher than that of the unloaded carrier. (Supported by NIH grant GM-31269.)

W-Pos264 RELATION BETWEEN THE CHLORIDE AND PHLORETIN BINDING SITES ON BAND 3. R. B. Gunn, L. Gilbert and O. Fröhlich. Department of Physiology, Emory University School of Medicine, Atlanta GA 30322.

The external aspect of the anion transporter, band 3, in red cells was probed with five related molecular structures by measuring their individual inhibition and their pairwise inhibition of Cl exchange. Individually, phloretin (P) is a noncompetitive inhibitor with a binding constant of 1.5 μM; 2,4,6-trihydroxyacetophenone (THA), which is one half of the phloretin molecule, is an apparent mixed inhibitor with K_i=74 μM and K'_i=40 μM; phloroglucinol (PG), a still smaller part of phloretin, inhibited with K_i=K'_i=7.1 μM. 4,4'-dinitrostilbene-2,2'-disulfonate (DNDS) is competitive, and m-nitrobenzenesulfonate (NBS), which is one half of a DNDS molecule, is competitive with K_i=550 μM. Cl exchange was then measured at Cl_o=22.5 mM as function of one inhibitor concentration, I_1, at four fixed concentrations of the second inhibitor, I_2. If the lines on the Dixon graph (J^{-1} vs. I_1) at the four fixed concentrations were parallel, the inhibitors were considered mutually exclusive; if they converged, the two inhibitors were considered to bind at the same time to separate sites. Mutually exclusive binding occurred between P and PG, P and NBS, P and DNDS, P and THA, PG and THA, and NBS and DNDS. Non-mutually exclusive binding occurred between THA and DNDS, PG and DNDS, THA and NBS, and PG and NBS. A strict steric interpretation of these data suggests separate chloride and phloretin binding sites which are simultaneously occupiable by DNDS or NBS, whereas PG and THA only occupy the phloretin sites. The small size of NBS would mean that the Cl and P sites are close. (Supported by NIH grants GM-30754, HL-28674 and GM-31269.)

W-Pos265 MEMBRANE ASSOCIATED AND AGE RELATED CHANGES IN HUMAN RED BLOOD CELL MEMBRANES MEASURED BY QUASI-ELASTIC LIGHT SCATTERING. Roy B. Tishler, The Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218

This report identifies further results of quasi-elastic light scattering (QELS) studies of the membrane/cytoskeleton (m/c) of human red blood cells (RBCs). The effects of specific alterations of the m/c on the QELS signal and changes associated with cellular age have been identified. Measurements of the intensity autocorrelation function, $g^{(2)}(\tau)$, were made using a QELS microscope spectrometer. The average correlation time, T_{corr}, of $g^{(2)}(\tau)$, determined by fitting the data with a single exponential, was used for comparing data from different cell populations. The QELS signal from RBCs has been previously shown to arise from spontaneous motion of the m/c (Tishler & Carlson, Biophys. J., (1986), 49:145a) Consequently, modifications of the RBC which alter the physical properties of the membrane or cytoskeleton would be expected to change the QELS signal. Increasing the cholesterol/phospholipid ratio of the RBC membrane led to an increase in T_{corr}. Cross-linking the cytoskeletal proteins by increasing the intracellular Ca^{++} concentration also led to an increase in T_{corr}. The effect of increasing NaCl concentration was previously interpretted in terms of osmotic changes. Further studies using salts which have cations that are permeable or non-permeable to the RBC membrane clearly identified an osmotic basis for the NaCl changes. Density centrifugation of RBCs was used to separate cells on the basis of age. As RBCs age, they become more dense and their membranes become less flexible. This change in the membrane would be expected to alter the QELS signal. The mean value of T_{corr} for older, denser RBCs was greater than that for the younger, less dense cells. Supported by USPH/NIH grant AM12803 to F. D. Carlson (Dept. of Biophysics, JHU) and 5T32GM07309 (R.B.T.)

W-Pos266 KINETICS OF NA/LI EXCHANGE IN HIGH K (HK) AND LOW K (LK) SHEEP RED CELLS (SRC). K.H.Ryu, N.C. Adragna*, D. Bradley, and P.K. Lauf. Depts. Physiology & Biophysics, and*Pharmacology and Toxicology, Wright State University School of Medicine, Dayton, Ohio, 45401-0927.

More than 90% of the ouabain-resistant Na flux in HK and LK SRC is mediated by the Na/Na homo-exchange (Tosteson & Hoffman, J. Gen.Physiol. 44:169,1960) the physiologic contribution of which to the steady state ion composition of both cell types is unclear. Although there are a few subsequent studies, no detailed analysis of the kinetic parameters of Na/Na exchange in sheep is available. In defining these properties in HK and LK SRC we adjusted cellular Na and Li levels by the nystatin method using choline as replacement ion. In LK SRC the maximum Li influx exchange rate (Vm) was 2.5 $\pm$0.5 mmoles/L.cell water x hr ($\pm$ SE,n=5) with an apparent $K_{\frac{1}{2}}$ of 0.63 $\pm$ 0.04 mM external Li concentration, $(Li)_o$. In HK SRC the Vm and $K_{\frac{1}{2}}$ values were 1.5 $\pm$ 0.1 (n=4) and 0.70$\pm$0.06, respectively. Corresponding values for Li efflux were: Vm = 1.5 $\pm$ 0.3 (n=5) and $K_{\frac{1}{2}}$ = 0.27 $\pm$ 0.05 in LK SRC, and 0.97 and 0.26 in HK (n=2). Vm and $K_{\frac{1}{2}}$ values for Na influx in LK SRC were 2.4 $\pm$ 0.5 and 14.0 $\pm$ 3.6 (n=3), respectively. When Na influx and Li efflux were measured simultaneously, saturation curves of Li efflux versus varying extracellular Na concentrations, $(Na)_o$, were obtained with a trans-stimulatory effect of $(Na)_o$ on Li efflux. The Vm and $K_{\frac{1}{2}}$ values for Li efflux vs Na_o were 1.41$\pm$0.3 mmoles/L.cell water x hr, and 12.0$\pm$ 1.4 mM $(Na)_o$, respectively. The transeffect of $(Na)_o$ on Li efflux was studied at different cellular Li concentrations. After correction for major Li leaks in choline or Mg media the ratio of the apparent Vm to $K_{\frac{1}{2}}$ values for Li seemed to be independent of $(Na)_o$. Such behavior has been reported for human red cells to be consistent with a consecutive or"ping-pong" mechanism of Na/Na(Li) exchange (Hannaert & Garay, J.Gen.Physiol. 87:353,1986). NIH grant 5 ROI-AM 37160 (to PKL).

W-Pos267 DETECTION AND QUANTITATION OF SUPEROXIDE RADICALS FROM ISCHEMIC REPERFUSED HEARTS. M.S. SALKA AND M. ASHRAF, DEPARTMENT OF PATHOLOGY, UNIVERSITY OF CINCINNATI, CINCINNATI, OHIO.

There is indirect evidence in the literature that cardiac ischemic reperfusion injury is largely mediated by oxygen derived free radicals where superoxide ($O_2^{\cdot-}$) anions are believed to be the precursors of other more damaging species. An assay for the detection and quantitation of superoxide from ischemic reperfused rat hearts was conducted. Hearts were cannulated, retrogradely perfused with an aerobic Krebs-Henseleit (K-H) medium for equilibration, then they were subjected to 40 minutes of global (zero flow) ischemia maintained at 37°C (n=6). Thereafter, the hearts were reperfused with the same aerobic K-H medium containing 50 µM of ferricytochrome C with or without the presence of 20 µgm per ml of superoxide dismutase (S.O.D.). The flow rate was maintained at 7.75 ml/min. The coronary effluent collected at five second intervals during the first minute and every 30 minutes thereafter, was assayed spectrophotometrically for reduced cytochrome C (R-cyt C) at 550 nm. $O_2^{\cdot-}$ production was quantitated as nanomoles per milliliter per gram heart weight of SOD-inhibitable reduced cytochrome C (R-Cyt C in the absence of SOD minus R-cyt C in the presence of SOD) plotted against total volume (V) of coronary effluent collected since the start of reperfusion. $O_2^{\cdot-}$ production was maximal (8 nanomoles per ml per gram heart weight) at 3 seconds (V=0.4 ml) and decreased dramatically to negligible values by 25 seconds of reoxygenation (V=3 ml). Control (non ischemic hearts) demonstrated negligible cytochrome C reduction. These data suggest that this assay may be valuable in studying the mechanisms of oxygen radical production by ischemic reperfused hearts. (Supported in part by a grant from Hariri Foundation).

W-Pos268 HEAVY WATER EFFECTS ON SOME CELLULAR PROCESSES

V. Vasilescu, Eva Katona, Mioara Tripşa, Ioana Bărbat, A. Dinu
Department of Biophysics, Medical Faculty, Bucharest, Romania

Systematical investigations on partial and/or quasi-total deuteration of muscle and nerve cells enabled to demonstrate proton involvement in the basic cellular processes. From data analysis the compartmentation of biosystems appeared as an essential characteristics of their structure. Heavy water effects on this compartmentation and on the function of various biosystems were followed up.

There were studied also the chronic effects of ~99% D_2O on cellular multiplication. New entities of life developped in quasi-total deuterated media have been observed and characterized.

Certain life processes in media containing deuterons substituted for protons are attempted to be modelled.

W-Pos269 VANADATE-STIMULATED NADH OXIDATION ACTIVITY ASSOCIATED WITH S. cerevisiae PLASMA MEMBRANES. Lori-ann E. Minasi and Gail R. Willsky, Dept. of Biochemistry, SUNY at Buffalo, 102 Cary Hall, Buffalo, NY 14214

Although vanadium has been shown to be a structural metal in an algal nitrogenase and bromoperoxidase, the precise role of vanadium in metabolism has not been ascertained. A vanadate-stimulated NADH oxidation activity has been reported in animal and liver plasma membrane fractions. We were interested in studying this activity in an organism well suited for genetic analysis. Therefore, vanadate-stimulated NADH oxidation activity was shown to exist in a crude membrane fraction obtained from the yeast S. cerevisiae. Cell membranes were obtained by differential centrifugation of a yeast cell homogenate made by using glass beads in a DYNOmill. Vanadate-stimulated NADH oxidation activity was not found associated with any fraction containing soluble proteins from the cytosol.

The cell membrane fraction was further separated into plasma membranes and mitochondrial membranes using glycerol gradient centrifugation. The vanadate-stimulated NADH oxidation activity was present in the plasma membrane fraction. A basal level of vanadate-independent NADH oxidation activity was associated with the mitochondrial membrane fraction. Maximum vanadate-stimulated NADH oxidation activity (720 nmol NADH oxidized/min/mg) occurred in the presence of 0.45 mM vanadate.

W-Pos270 PATCH CLAMP ANATOMY: HIGH VOLTAGE ELECTRON MICROSCOPY OF INVIVO PATCHES. F.Jung, M.J. Song* and F. Sachs, Biophysics, SUNY, Buffalo, NY and *Wadsworth Laboratories, NYS Dept. Health, Albany, NY.

Although a great deal of work has been done on the electrophysiology of membrane patches, little is known about the structure of the patch. In order to view the structure of a patch "in-vivo" , i.e. in the pipette, we have used high voltage electron microscopy to examine excised patches. Inside-out patches from chick myocytes are formed in a unfirepolished borosilicate pipette. The tip is kept under saline and attached to an EM grid using fast cure epoxy. The pipette barrel is broken off leaving about 1mm of tip attached to the grid. This tip may then be fixed and stained. The grid is then either quick frozen in liquid N_2 and freeze dried or critical point dried with actone and CO_2. In the 1.2MV microscope, the pipette is transparent for about 20 microns from the tip. Useful magnifications up to net 300,000 can be obtained in the tip region. Preliminary results indicate that the inside out patch must be considered as a "bud" containing cytoskeleton and cytoplasm rather than a piece of "pure membrane". It appears quite feasible to use gold labelled antibodies to study the histochemisty of the patch. Studies of outside-out patches are in progress.

Supported by NIADDK DK37792-091 and USARO 22560-LS

W-Pos271 IONIC PROPERTIES OF THE SUGAR BEET ROOT VACUOLE MEMBRANE.

L. Coyaud(1), A. Kurkdjian(2), R. Hedrich(3) and R.T. Kado(1). Laboratoire de Neurobiologie Cellulaire et Moléculaire(1), Laboratoire de Physiologie Cellulaire Végétale(2) CNRS, Gif sur Yvette, France and Pflanzenphysiologishes Institut Universität Göttingen, Göttingen, W.Germany(3).

The vacuole is a membrane bound organelle occupying most of the intracellular space of plant cells. Its function is regulatory, sequestering and releasing ions, metabolites, organic acids and water. While it is well known that the regulation is accomplished by the movement of these substances across the vacuolar membrane, no direct evidence has been available until only recently. It has now been shown that the membrane of the barley vacuole has ion channels and an ATPase which pumps H^+ ions into the vacuole (Hedrich et.al. FEBS Lett. 204, 228 1986).

We have studied the membrane properties of the sugar beet root vacuole using the patch-clamp technique. This membrane has so far shown two kinds of channels. A predominant large current channel and a less frequent one of about one fifth the conductance. our report concerns only the large channel observed in whole-cell and outside-out patch configurations and an ATP-activated current in the whole-cell configuration.

The channel opening was very voltage dependent, showing almost no openings for potentials more positive than -20 mV inside to outside. When open, its conductance was 70 pS and 180 pS with symmetrical 50 and 200 mM KCl solutions respectively. The permeability ratios pK/pi for the ions tested were: Na^+=1 , Cl^-=6 , $Malate^{2-}$=5.1 , NO_3^-=3.2 , Acetate =2.7.

In the whole cell configuration, applying 5 mM ATP in the bathing medium produced a current of about 60pA which could be sustained for many minutes and disappeared with wash-out of the ATP. In current clamp, the ATP produced a potential shift of up to +55 mV. These effects are believed to be due to the pumping of H^+ ions.

Our present results show that the vacuolar membrane is well adapted to the patch clamp technique and in view of its limited channel diversity, may lend itself very well to the study of transport mechanisms.

W-Pos272 OPTIMAL ISOLATION CONDITIONS FOR ION TRANSPORT STUDIES IN PIG AORTIC ENDOTHELIAL CELLS.
NC Adragna, Wright State Univ., School of Medicine, Dept. Pharm/Tox, Dayton, OH 45435.

Endothelial cells (EC) are highly susceptible to injury as reflected in rapid changes of the normal steady state cation composition. Thus, for ion transport studies it is necessary to assess different methods of isolation and culture conditions to establish their suitability to preserve cell integrity as well as to provide an optimal yield. As a first step in this attempt, the effect of 3 factors was studied on cell count and viability in freshly isolated EC. The method by Gimbrone et al (TCA Manual, 4:813, 1978) was used as a reference. 1). Change in bivalent cations (Ca and Mg) in the isolation medium for collagenase treatment (control, 1mg/ml, 40 min.). Mg alone (0.5mM) gave the highest viability when compared to the control containing the two bivalents ($\bar{x}$ ± SE)(93.8±1.0 vs 74.2 ± 3.2%, respectively, $p<0.01$). The lowest viability was obtained in the presence of the two bivalent chelators EDTA+EGTA (0.05% each), (53.3+2.6%, $p<0.01$). In addition, a significantly higher number of cells and protein content per aorta ($p<0.05$ and $p<0.001$, respectively) was obtained with Mg alone. In Ca alone (0.9mM), the number of cells/µg protein was higher ($p<0.05$) than in Mg alone suggesting that cell shrinkage has occurred in the presence of Ca without Mg. These results indicate that Mg but not Ca is necessary during collagenase treatment. 2). Optimal incubation time with 1mg/ml collagenase was determined using 5,15,40 and 60 min incubations. Sixty minutes gave the best yield with no change in viability with respect to the control. 3). Optimal concentration of collagenase was determined using 0.1,0.3,0.5 and 1mg/ml. The highest viability was observed with 0.3mg/ml with no difference in the yield. It is concluded that 0.5mM Mg, 60 min and 0.3mg/ml collagenase provide the best conditions in terms of viability and yield. Supported by WSU Seed grant and Miami Valley Heart Chapter, AHA MV-86-01.

W-Pos273 ENDOCYTOSIS OF LIPOSOMES BY MACROPHAGES. David L. Daleke, Keelung Hong, and Demetrios Papahadjopoulos, Cancer Research Institute and Department of Pharmacology, University of California, San Francisco, CA 94143.

Using a recently developed fluorescent technique to monitor continuously the uptake of liposomes by cells we have followed the endocytosis of lipososmes by macrophages. Pyranine (hydroxypyrene trisulfonate), a highly fluorescent, water soluble, and pH sensitive dye, is encapsulated at high concentration into the lumen of large unilamellar vesicles. The excitation spectrum of this dye exhibits a strong pH dependence, especially in the range 6.0-7.5. Thus, the uptake of liposomes by cells into acidified endosomes or secondary lysosomes (pH < 6) is monitored through alterations in the excitation spectrum. The percent of dye at low pH is determined by a comparison of fluorescence emission intensity at two different excitation wavelengths. The intra- and extracellular distribution of liposomes and their approximate pH is detected by fluorescence microscopy. The kinetics of liposome uptake, and a measure of the amount of liposomes incorporated at low pH is made with a fluorometer, either with cells in suspension or with a monolayer of cells grown on a glass coverslip.

This technique is now applied to the study of liposome endocytosis by a variety of mouse macrophage and macrophage-like cell lines: thioglycolate-stimulated mouse peritoneal macrophages, and the macrophage cell lines RAW 264.7, J774, and P388D1. These cells incorporate 30-75% of the dye encapsulated in negatively charged liposomes into low pH compartments within 60 min at 37°C. Lysosomotropic agents and protonophores which raise the pH of intracellular compartments also change the spectral characteristics of the incorporated dye. The rate and extent of incorporation is dependent on temperature, the metabolic state of the cell, and cell type; peritoneal macrophages and J774 cells being the most efficient. This technique now allows detailed studies of the kinetics of liposome endocytosis and the fate of endocytosed liposomes. Supported by NIH grants GM 28117 and CA 35340 and ACS grant PF2774.

W-Pos274 FLUID VISCOSITY DOMINATES HAIR-BUNDLE DAMPING IN TRANSDUCER CELLS OF THE FROG SACCULUS.
W. Denk and W. W. Webb, Applied Physics, Cornell University, Ithaca, NY 14853

We have measured the time varying spontaneous displacements (Brownian motion) of the tips of the sensory hair bundles of transducer cells in an isolated saccular epithelium of bullfrogs, Rana catesbeiana (Hudspeth, Ann. Rev. Neurosci. 6, 187, 1976). Our confocal interferometry method is based on Differential Interference Contrast microscopy with focused laser illumination and a position sensing silicon quadrant detector. It provides sensitivity of a few picometers/$\sqrt{Hz}$ (W. Denk et al., Biophys. J. 49, 21a, 1986). The observed rms displacement amplitudes (3-6 nm) are consistent with bundle stiffness values measured by the fiber probe method (Ashmore, J. Physiol. 350, 20P, 1984). The power spectra of many hair cells show two distinct regions, a gradual power law drop with frequency exponents of about 0.6 to 0.9 for the low frequency region, and above the roll off frequency (200-600 Hz) a drop with an exponent significantly steeper than -2. An increase in viscosity of the medium which replaces the endolymph was achieved by adding a high molecular weight sugar. The viscosity increase changes the spectra as expected for damping of the hair bundle by the surrounding fluid; the knee frequency decreases, while the rms amplitude stays unchanged. The slope in the high frequency region decreases to -2, probably because the viscous penetration depth becomes larger than the hair bundle length over the entire measured frequency range.

Supported by NIH (GM33028)

W-Pos275 COLLISIONAL CONTROL OF FACTOR Va LIGHT-CHAIN (Va-LC) BINDING TO VESICLES.
Alan J. Abbott and Gary L. Nelsestuen Dept. of Biochemistry, Univ. of Minnesota, St. Paul, MN 55108.

When a protein collides with a large particle (a cell or vesicle) and binds in a collisionally efficient manner, the limiting step in the association is the collision. The rate constant for collision, k_{coll}, is proportional to the sum of the radii of the particles (effectively equal to that of the large particle) and to the sum of their diffusion constants (effectively that of the protein). Furthermore, N collisions are required to fill a large particle with N receptors. The observed rate constant for filling receptors becomes: $k_{app}=k_{coll}/N$.

Va-LC (M.W.=80kD.) binding to phospholipid vesicles (20% PS, 75% PC, 5% PE-dansyl) was studied and monitored by fluorescence energy transfer methods. The association properties indicated a collisionally efficient reaction. In this case, the functional receptor number per vesicle (N) was proportional to vesicle surface area ($4\pi R_v^2$) so that $k_{app}=k_{coll}/(4\pi R_v^2/A)$, where A is the area per binding site. The observed association rate constant (k_{app}) was proportional to $1/R_v$. A plot of k_{app} vs. $1/R_v$ gave a slope that was a function of A and two known constants: $k_{app} = (D_l N_{AV}/1000)*R_v^{-1}*A$. The association rate constant for all vesicles used was close to the calculated collisional rate constant. The Arrhenius Activation energy was 4-5 kcal/mole.

The Va-LC dissociation rate should display a similar effect ($k_{off} \propto 1/R_v$) due to efficiency of recapture of dissociated but adjacent proteins through microcollisions. However, k_{off} is also a function of the intrinsic protein-receptor affinity: $k_{off} = k_{(-)coll} * K_{intrinsic}$. This intrinsic affinity for Va-LC was found to vary widely with vesicle size as indicated by disparate activation energies for dissociation. It is uncertain whether k_{off} was influenced by the diffusional parameters. Other effects are possible and will be discussed. In agreement with these rate data, the overall equilibrium binding constants for Va-LC to small vs. large vesicles were quite different and the relative affinities changed dramatically with temperature.(Supported by grant No. HL 15728.)

W-Pos276 INTERACTION OF AMIODARONE WITH THE CARDIAC MUSCARINIC RECEPTOR IN VITRO. ROBERT A. COLVIN, JOSEPH OIBO, LARRY TYLER, AND DAVID LEAK. DEPT. OF PHARMACOLOOGY AND INTERNAL MEDICINE, ORAL ROBERTS UNIVERSITY SCHOOL OF MEDICINE, TULSA OK 74137.

We studied the interaction of amiodarone hydrochloride (cordarone), a potent antiarrhythmic drug, with the muscarinic receptor in purified canine cardiac sarcolemmal vesicles, by its ability to inhibit equilibrium binding of the antagonist ^{3}H-Quinuclidinyl benzilate (QNB). At 0.2nM, QNB binding was inhibited by amiodarone with an IC_{50} of 5.5×10^{-7}M. This concentration would represent a clinically relevent serum concentration of less than 1 ug/ml. Scatchard analysis of QNB saturation isotherms (37°C, pH 7.4) in the presence of 1 uM amiodarone showed an increase in dissociation constant over control from .045 $\pm$ .002nM to .084 $\pm$ .001nM while maximal binding capacity was unaffected, 10.8 $\pm$ 1.14 and 10.5 $\pm$ 1.48 pmole/mg (mean $\pm$ SEM n=3) respectively. The inhibitory effect of amiodarone on equilibrium binding was highly dependent on the drug:membrane lipid mole ratio with effects beginning at a ratio of less than .1:1. Hill plot analysis was consistent with the interaction of QNB at a single site in the presence or absence of amiodarone. Amiodarone (1uM) stimulated the rate of association of QNB (0.1nM) with the muscarinic receptor as evidenced by an increase in k_{obs} from 4.65 $\pm$ .65 to 5.65 $\pm$ .80 $\times 10^{-4}s^{-1}$ n=4. At all concentrations of QNB tested, the presence of 1uM amiodarone from the start of the binding reaction had no observable effect on the rate of dissociation of QNB as measured by the addition of 100nM atropine at equilibrium. The results therefore suggest that at clinically relevent concentrations, amiodarone may act to modulate muscarinic receptor affinity and appears to alter preferentially unoccupied muscarinic receptors.

W-Pos277 LOW DENSITY LIPOPROTEIN (LDL) RECEPTOR DYNAMICS ON CELL SURFACES. Richik N. Ghosh and Watt W. Webb, Applied and Engineering Physics, Cornell University, Ithaca, New York 14853.

Quantitative digital video fluorescence microscopy is used to study LDL receptor dynamics on the surface of human skin fibroblasts. Clusters and single molecules of LDL receptor are tracked using the intensely fluorescent ligand diI-LDL (Barak and Webb, J. Cell Biol. 90, 595-604, 1981). A panoply of effects is observed on (1) normal fibroblasts (GM3348) treated with the calmodulin inhibitor Stelazine to prevent internalization (Ghosh, Gross and Webb, Biophys. J. 49, 313a, 1986); and on (2) internalization deficient "J.D." fibroblasts (GM2408A). LDL receptors underwent both random and directed (i.e., nonrandom correlated) motion. A variety of directions on the same cell were observed for those receptor molecules having directed tracks, including trajectories towards, away from, and parallel to the nearest cell edge. Several areas on cell surfaces had neighboring particles with closely correlated motion, both in their directions and in the time intervals of their major motions. A majority of the directed receptor molecules on a Stelazine treated cell were found to be quiescent for most of the observation time, but "came alive" at a certain instant and underwent a highly correlated series of virtually parallel steps before resuming their nearly quiescent state. To date, no correlation between particle aggregate sizes and their mobility has been found.

Supported by grants from the NSF (DMB 83-03404), ONR (N00014-84-K-0390), NIH (GM33028) and the Cornell Biotechnology Program (which is sponsored by the N.Y. State Science and Technology Foundation and a consortium of industries).

W-Pos278 STRUCTURAL ANALYSIS OF NICOTINIC ACETYLCHOLINE RECEPTOR: POSITION OF CYTOSKELETAL ELEMENTS. Michael P. McCarthy, Alok K. Mitra, and Robert M. Stroud. Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

We have performed three-dimensional structural analysis of ordered arrays of the nicotinic acetylcholine receptor (AChR) from Torpedo californica both in the presence and absence of cytoskeletal elements. Tilted, negatively-stained images of ordered vesicles were obtained in the electron microscope under low dosage exposure conditions. The quality of the individual ordered vesicles was determined by optical diffractometry. Images of the best ordered vesicles at various tilt angles were digitized, and Fourier analyzed. The phases and amplitudes of a number of ordered vesicles were combined and three-dimensional contour maps of the AChR were generated, both in native vesicles and after removal of cytoskeletal elements (specifically the 43 K protein). Preliminary results indicate that, in native vesicles with the 43 K protein present, consistent and fairly-well defined density is observed between and beneath AChR monomers on the cytoplasmic side of the bilayer which is not observed in vesicles with the cytoskeletal elements removed. This observation is consistent with the expected positioning and stoichiometry of the 43 K protein with respect to the AChR.

W-Pos279 TERBIUM LUMINESCENCE STUDIES OF ADRIAMYCIN BINDING TO GH3/B6 PITUITARY TUMOR CELLS. Robert G. Canada. Department of Physiology and Biophysics, Howard University College of Medicine, Washington, D.C. 20059.

The luminescent properties of terbium (Tb^{3+}) were used to study the binding of adriamycin to GH3/B6 pituitary tumor cells. Clinically relevant concentrations of adriamycin were found to quench the luminescence intensity of the Tb^{3+}-GH3/B6 complex. The IC_{50} for adriamycin inhibition of Tb^{3+}-GH3/B6 luminescence was 1.0 μM. Eadie-Scatchard analysis revealed that the affinity of Tb^{3+} for the GH3/B6 cell was substantially increased in the presence of adriamycin; in that, the apparent dissociation constant for the Tb^{3+}-GH3/B6 complex in the presence of adriamycin (K_d = 8.7 μM) was less than that in the absence of adriamycin (K_d = 22.1 μM). Inspection of the Tb3+-GH3/B6 emission spectrum and the visible absorption spectrum of adriamycin strongly suggests that the quenching of Tb^{3+} luminescence by adriamycin was due to a dipole-dipole resonant energy transfer mechanism. The actual distance of separation between the bound probe and toxin was calculated to be 39.7 Å. The data suggests that adriamycin can interact with a specific Ca^{2+} binding protein in the plasma membrane of tumorigenic cells.

W-Pos280 ISOLATION OF LSD-BINDING PROTEINS FROM THE BOVINE BRAIN BY AFFINITY CHROMATOGRAPHY. Timothy K. Gallaher and Howard H. Wang, Department of Biology, University of California, Santa Cruz, CA 95064.

The psychotomimetic compound, D-lysergic acid diethylamide (lysergide, LSD) is a competitor for serotonin (5-hydroxytryptamine, 5-HT) and for dopamine (3-hydroxytyramine, 3-HT) binding sites in brain tissues. We report the use of a LSD-like ligand for the isolation of dopaminergic and serotonergic receptors from solubilized preparations of bovine brain tissue. The ligand lysergic acid ethylamidoethylbromide was synthesized and coupled to a sulfhydryl agarose gel for affinity chromatography. Detergent solubilized (3% Triton X-100/1% Tween 80) membrane fragments of bovine frontal cortex were applied to the affinity column which was eluted with a detergent solution until protein concentration reached a base level. The column was then eluted with a 5-hydroxytryptamine solution (156 μM) with the fractions saved and dialyzed. Assay of the dialyzed preparation showed ^{3}H-5-HT binding activity of 48 pmol/mg protein. In separate experiments, the crude membrane preparation was solubilized in 1.25% CHAPS, purified by affinity chromatography in the presence of soybean lecithin (Asolectin), and reconstituted in lipid membranes by dialysis in the presence of Asolectin. Reconstituted membranes were assayed for the ^{3}H-5-HT binding activity. These reconstituted receptor membranes exhibited a ^{3}H-5-HT binding specific activity of 237 pmol/mg of protein and an apparent K_d of 161 nM. The specific activity represents an enrichment of about 2000 times over that of the crude membrane preparation.

In affinity columns where the 5-HT-binding protein has already been eluted, further elution of the affinity column with a 3-hydroxytyramine solution (180 μM) yielded a protein sample which showed on SDS-gel electrophoresis and with silver staining a broad band indicating an apparent molecular weight of 126 to 149 kD. We suggest that the LSD-affinity column may be useful for the purification of both serotonergic and dopaminergic receptors.

W-Pos281 COMPETITION OF ALCOHOLS WITH LOCAL ANESTHETICS FOR ACETYLCHOLINE RECEPTOR BINDING SITES. Kathryn M. Behner and Howard H. Wang, Department of Biology, University of California, Santa Cruz, CA 95064

Noncompetitive antagonists inhibit the action of acetylcholine (Ach) without preventing its binding. Such antagonists stabilize the receptor in its desensitized state and induce high affinity binding of Ach. Local anesthetics are a type of noncompetitive antagonists which inhibit the action of Ach by blockage of the ion channel. They are usually positively charged amines which are either fully or partially charged at physiological pH. General anesthetics are another type of noncompetitive antagonist. However, they are uncharged and hydrophobic molecules, and their mechanism of action is unknown. In our laboratory we have investigated the possibility that local and uncharged anesthetics share the same binding site and therefore the same mechanism of action. To test this hypothesis we have used a spin-labeled local anesthetic analogue, C6SLMeI. It has been established through both ESR and radioligand binding studies that C6SLMeI competitively inhibits phencyclidine binding (*Mol. Pharmacol.*, 30:243, 1986). A variety of alcohols were used as uncharged anesthetics. Boyd and Cohen (*Biochemistry*, 1984, 23:4023) have determined that incubation of membrane suspensions with aliphatic alcohols (1-propanol, 2-propanol, 1-butanol, and tert-butyl alcohol) and with benzyl alcohol produced a dose-dependent increase in the amount of receptor in a high affinity conformation. Several alcohols at various concentrations were individually incubated with an AchR preparation (0.8 nM) in the presence of Carb (5 mM) after initial incubation in C6SLMeI (3 μM). The alcohols included benzylalcohol (0.1-100 mM), octanol (0.1-10 mM), ethanol (0.01 mM-1 M), propanol (0.01-100 mM), 1-butanol (1-500 mM) and t-butyl alcohol (0.01-100 mM). The incubation solutions were centrifuged and evaluated by electron spin resonance. The effect of the alcohol on the spin labeled local anesthetic was compared to a control with no alcohol present. In the case of each alcohol, the immobilized component decreased as the concentrations of the alcohol increased. Thus the amount of bound C6SLMeI appeared to be reduced due to competition with the alcohols.

W-Pos282 DECAY ACCELERATING FACTOR DIFFUSES RAPIDLY ON $HeLa_{AE}$ CELL SURFACES. James Thomas and Watt Webb, Applied Physics, Clark Hall, Cornell University, Ithaca, NY 14853; Michael A. Davitz and Victor Nussenzweig, Department of Pathology, New York University School of Medicine, New York NY 10016.

Decay Accelerating Factor (DAF) is a 70 kDal membrane protein that inhibits complement cascade amplification with as few as 70 DAF molecules on a single erythrocyte (Medof et al., J. Exp. Med. 160, 1558, 1984). Recent studies have established that DAF is anchored to the plasma membrane via phosphatidylinositol (Davitz et al., J. Exp. Med. 163, 1150, 1986); thus both its function and structure suggest rapid diffusibility. Fluorescence Photobleaching Recovery (FPR) was used to measure the mobility of DAF on $HeLa_{AE}$ cells; the DAF was labeled with fluoreceinated IA10 anti-DAF monoclonal antibody. A mean diffusion coefficient of $1.61\pm0.17\times10^{-9}cm^2/s$ was obtained, an order of magnitude faster than most cell surface proteins on vertebrate cells in culture. The lipid analog DiI-C_{18} was found to diffuse at $2.44\pm0.40\times10^{-9}cm^2/s$, slightly slower than the usual. The cell surface glycoproteins labelled by rhodamine-succinyl-concanavalin A diffused at $0.18\pm0.08\times10^{-9}cm^2/s$. Repeated bleaching of a single spot yielded a limited range of values for D, while cell to cell variation was quite large. Typically, the mobile fraction was much less than one and significant diversity of mobility in the DAF molecule population is possible. Rapid diffusion of DAF may be a consequence of its lipid attachment morphology, but we have yet to understand the control and variability of that mobility.

Supported by grants from NSF (DMB 8303404) and ONR (N00014-84-K-0390) (J.T. and W.W.) and NIH (AI08499 (W.V. and M.A.D.).

W-Pos283 AN ISOLATED MEMBRANE PREPARATION FROM GIANT BARNACLE MUSCLE FIBERS. Aníbal A. Altamirano and John M. Russell. Dept. of Physiology & Biophysics, U.T.M.B. Galveston, TX 77550.

A plasma membrane-enriched preparation was obtained from muscle cells of the giant barnacle (B. nubilus) by homogenization, differential centrifugation, and centrifugation on a continuous (20-50%) sucrose gradient. After this step, the contents of the centrifuge tube were divided as follows: a light fraction (F1) at the 10-20% interface; a second, larger fraction (F2) between 20 and 40% sucrose, and a third fraction (F3) at about 45% sucrose. The pellet was also recovered. F1 was enriched in (Na,K)-ATPase. Ouabain-binding, determined by rapid filtration through cellulose-acetate filters was enriched 6 times (27.5±5.0 pmoles/mg, n=5), compared to that in the crude pellet fraction (5.24±1.09 pmoles/mg, n=8) and saturated with increasing ouabain concentrations ($K_{\frac{1}{2}}$ ∿ 0.2 µM). The activity of a mitochondrial marker in F1, succinate dehydrogenase (SDH), was 7 times smaller (0.56±0.2 µmoles/mg•sec, n=8) than in the homogenate (3.1±0.1 µmoles/mg•sec, n=3). SDH activity was higher in F2 (1.39±0.25 µmoles/mg•sec, n=8), F3 (1.52±0.32 µmoles/mg•sec, n=6) and the pellet (2.13±0.13 µmoles/mg•sec, n=6). Oxalate-supported Ca^{++} uptake and Ca^{++}-ATPase activities (sarcoplasmic reticulum markers) were low in F1 (0.28±0.06 nmoles/mg•sec, n=4, and 1.74±1.23 nmoles/mg•sec, n=3, respectively), but were significantly higher in F2 and F3. DIDS does not cross plasma membranes but binds to membrane proteins; this property was used to provide additional evidence that Fraction F1 is enriched in external membrane (sarcolemma). In membrane preparations using fibers previously labeled with $^3H_2$-DIDS (20 µM in artificial sea water, 1 hour at room temperature), F1 was enriched 7-fold in $^3H_2$-DIDS activity with respect to the crude pellet. This compares well with the enrichment noted for [^{3}H]-ouabain binding. From these data we conclude that F1 contained predominantly sarcolemmal membranes. Supported by DHHS NS-11946.

W-Pos284 DETERMINATION OF INTRACELLULAR PH OF BALB/c 3T3 FIBROBLASTS USING THE FLUORESCENCE OF 8-HYDROXY PYRENE- 1,3,6-TRISULFONIC ACID (PYRANINE). R.J. Gillies and K.A. Giuliano; Dept. Biochemistry; Colorado State Univ.; Ft. Collins, Colo. 80523

Changes in intracellular pH are associated with a number of cellular responses, such as proliferation (1). For measuring pH, intracellularly trapped, pH-dependent fluorescent probes are advantageous in terms of accuracy and sensitivity. In this communication, we describe the measurement of intracellular pH using pyranine that has been scrape-loaded into cells (2). Pyranine dye has an emission maximum at 514 nm. Its excitation is pH sensitive, with an isosbestic point at 415 nm and peaks at 405 nm and 465 nm which decrease and increase with pH, respectively. The 465/405 ratio can be used to monitor the pH, while the fluorescence at 415 nm indicates the total dye remaining in the cells. We have observed scrape-loaded dye to persist in cells for periods up to 8 hours. We have calibrated this dye in situ using nigericin/high K+, and have found that the pKa of the dye in situ is 7.82, as compared to 7.68 in vitro . We have observed that SITS lowers steady state intracelluar pH, yet does not inhibit the cells' ability to regulate pH in respose to alkaline or acid load. We have also observed that the pH of serum-depived cells is significantly lower than the pH of serum-conditioned cells in the absence of serum. (1) Moolenaar, WH (1986) Ann. Rev. Physiol. 48, 363-376 (2) McNeil, PL et al. (1984) J. Cell Biol. 98, 1556-1564

W-Pos285 THE STRUCTURE OF ZYMOGEN GRANULE MEMBRANE AND ITS RELATION TO GRANULE STABILITY AND FUNCTION. A. Pande, A.U. Freiburghaus, R. Ammann
Department of Medicine; University Hospital; Zürich (Switzerland)

High sensitivity differential scanning calorimetry and Fourier transform infra-red spectroscopy of zymogen granule membranes show two endothermic transitions in the temperature range between 5° C and 70° C. They are due to lipidic components of the membrane and are reversible. The first transition, centered around 45° C has been assigned to the gel-to-liquid-crystalline phase change, and the second, centered around 60 ° C, is tentatively assigned to the formation of micellar structures. This thermotropic behavior is rather unusual for a eucaryotic membrane, but may be a common feature of secretory granule membranes. Dynamic light scattering measurements show that the intact granules are stable at temperatures below the first transition and lyse rapidly above it. Investigations are underway to determine the effect of intracellular messengers on the phase behavior of these membranes and thermal stability of the intact granules. The stability of these granules is physiologically relevant; an attempt will be made to correlate the stability of the granules with the structure and composition of their membranes.

W-Pos286 ACTIVATION OF LYMPHOCYTE Na^+/H^+ EXCHANGE BY CONCANAVALIN A IS NOT MEDIATED BY INCREASED CYTOPLASMIC FREE Ca^{2+} OR ACTIVATION OF PROTEIN KINASE C. S. J. Dixon, C. Rowatt and S. Grinstein. Department of Cell Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada M5G 1X8.

Application of the mitogenic lectin, concanavalin A (Con A), to thymocytes rapidly stimulates phosphoinositide turnover (increasing the levels of inositol 1,4,5-trisphosphate and diacylglycerol), increases intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), and activates Na^+/H^+ exchange. The purpose of this study was to determine whether the Con A-induced stimulation of the Na^+/H^+ antiport is secondary to increased $[Ca^{2+}]_i$ or activation of protein kinase C by diacylglycerol. Na^+/H^+ exchange activity was measured by monitoring the fluorescence of rat thymocytes loaded with the pH-sensitive dye, bis(carboxyethyl)carboxyfluorescein (BCECF). Con A induced a rapid, amiloride-sensitive, Na^+-dependent increase in cytosolic pH (pH_i) ($\Delta pH = 0.12 \pm 0.04$). To test the role of $[Ca^{2+}]_i$, cells were loaded with both BCECF and quin2, a fluorescent intracellular Ca^+ indicator with a large capacity for buffering cytoplasmic Ca^{2+}. In Ca^{2+}-free medium, Con A induced no change in $[Ca^{2+}]_i$ but activation of Na^+/H^+ exchange was still observed ($\Delta pH = 0.19 \pm 0.06$). In addition, a dose (50 nM) of the Ca^{2+} ionophore, ionomycin, sufficient to increase $[Ca^{2+}]_i$ to the level induced by Con A, failed to induce a comparable activation of Na^+/H^+ exchange. These data suggest that activation of the antiport by Con A is not mediated by changes in $[Ca^{2+}]_i$. We next examined the possible role of protein kinase C. Incubation of thymocytes overnight with 10^{-7} M 12-O-tetradecanoylphorbol 13, acetate (TPA) has been shown previously to downregulate protein kinase C activity to negligible levels. Following this treatment, Na^+/H^+ exchange was no longer activated by the phorbol ester TPA, but was, however, still stimulated by Con A. We conclude that Con A-induced activation of Na^+/H^+ exchange involves a 2nd messenger other than $[Ca^{2+}]_i$ or protein kinase C. (Supported by the Medical Research Council of Canada)

W-Pos287 EFFECT OF CYCLOSPORIN A AND IONOPHORES ON THE INTRACELLULAR PH OF LYMPHOCYTES AS MEASURED BY FLOW CYTOMETRY.

A. Aszalos, S. Damjanovich, and L. Tron (Introduced by ATTILA SZABO) Division of Drug Biology, Food and Drug Administration, Washington, D.C. 20204 and Department of Biophysics, Medical University School, Debrecen, Hungary.

A flow cytometric method was developed to measure intracellular pH based on the observation of fluorescence intensity of 2',7'-bis-(carbocyethyl)-5,6-carboxy-fluorescein loaded cells at 530nm and 610nm. Comparison of the sensitivity of intracellular pH measurements using single wavelength (530 nm) or fluorescence intensity ratio (530nm/610nm) demonstrated that single wavelength measurements were more sensitive in the pH range of 6.5-7.8.

We have found that Ca^{2+} ionophore, A23187 caused no detectable intracellular pH change in either mouse spleen or human peripheral blood lymphocytes. The ionophores nigericin, CCCP and monensin caused intracellular pH changes consistent with their known properties as ionophores.

The effect of Cyclosporin A (CsA), an immunosuppressor drug, on the intracellular pH of resting lymphocytes was investigated using the above method. CsA had no effect on intracellular pH at pharmacological doses (1ug/ml). However, at higher doses, 3 or 10 ug/ml CsA caused a dose dependent reversible or permanent acidification.

W-Pos288 CALCIUM METABOLISM DURING AND AFTER CHOLINERGIC STIMULATION OF GASTRIC PARIETAL CELLS.

P.A. Negulescu and T.E. Machen, Physiology-Anatomy, Dept, University of California, Berkeley, 94720

Microspectrofluorimetry was used to measure intracellular [Ca], $[Ca]_i$, in single parietal cells of isolated rabbit gastric glands loaded with the calcium sensitive dye fura-2. Carbachol (Carb, 100 uM) caused a rapid elevation of $[Ca]_i$ from a resting level of 98 nM to 825 nM. This increase occurred equally well in Ca-containing, Ca-free or Ca plus 0.1 mM La solutions. Following the peak, $[Ca]_i$ decreased rapidly to a lower level which was somewhat elevated (200 nM) over baseline. This plateau phase persisted until either: Carb or Ca_o was removed or atropine (1 uM) was added. Repeated stimulations with carb caused characteristic, repeated increases of $[Ca]_i$ if Ca_o was present between exposures to carb. Repeated stimulations in La-containing or Ca-free media caused $[Ca]_i$ to rise during the first stimulation but not during subsequent stimulations. These results suggest: 1) Carb releases Ca from an intracellular pool as well as activating a Ca entry mechanism. 2) Ca released from the pool is rapidly extruded across the plasma membrane, but a small amount of Ca continually enters from the outside. 3) Removal of carb or treatment with atropine allows Ca_o to accumulate in the intracellular store through a La-sensitive mechanism. 4) Reloading of the store, which does not cause a detectable increase in $[Ca]_i$, is through a different mechanism than that which is responsible for the plateau during stimulation.

(Support: NIH AM-19520)

W-Pos289 MODELS OF INFORMATION PROCESSING IN MICROTUBULES, SR Hameroff, RC Watt, CW Schneiker, Advanced Biotechnology Lab, Optical Sciences Center, Univ. of Ariz., Tucson, Arizona

Cell interiors are organized by a dynamic cytoskeleton comprised of microtubules (MT), actin and intermediate filaments, and a microtrabecular lattice. MT and their supra-assemblies (centrioles, cilia, etc.) are involved in a wide range of important biological functions which involve apparent "real time" information processing. We have reviewed the literature to document at least eleven published theoretical models of dynamic information processing in MT. These include:

Atema-propagated conformational changes along tubulin subunits in sensory cilia-transduction at cell membrane

Albrecht-Buehler-"intelligent cytoplasm"-coupled conformational states among tubulin subunits-centrioles as directional signal detectors

Barnett-"string transformations" and translation among parallel arrayed microtubules

Bornens-MT network of coupled dipoles-centriole as "gyroscopic oscillator"

Conrad and Liberman-cytoskeleton as mechanical stretch transducers coupled to membrane

de Brabander-organizational function of MT-labeling of individual tubulin subunits by tyrosylation and immunofluorescence as coding mechanism

del Giudice-self focusing of electromagnetic energy into MT and filaments-solitons in MT

Hameroff and Watt-MT cellular automaton based on tubulin dipole coupling

Jarosch-contractile actin induced MT torque

Koruga-crystallographic symmetry of MT

Roth and Pihlaja-"gradionation" of tubulin subunit conformation representing patterns

The models are not mutually exclusive and lend insight to the informational capabilities of MT.

W-Pos290 CHANGES IN INTRACELLULAR pH REGULATION UPON DIFFERENTIATION OF HL60 CELLS. D. Restrepo, D. Kozody and P.A. Knauf. Dept. of Biophysics, Univ. of Rochester Med. Ctr., Rochester, NY 14642

We have studied the effects of phorbol 12-myristate 13-acetate (PMA), ionomycin (ION) and osmotic shock (OS) on intracellular pH homeostasis in HL60 cells using BCECF. pH_i is 6.88±0.03 in the undifferentiated (U) cell, 6.90±0.11 in the neutrophil-like (N) cell (1.25% DMSO 5 to 6 days) and 6.85±0.16 in the monocyte-like (M) cell (10nM 1,25-dihydroxyvitamin D_3 5 to 6 days). All three kinds of HL60 cells possess a Na^+-dependent H^+ extrusion mechanism inhibited by dimethylamiloride (DMA 20uM). A transport system inhibited by H_2DIDS (1mM) is also involved in pH_i homeostasis. Upon addition of DMA and H_2DIDS, pH_i falls to 6.5-6.7 (pH_o 7.40). In the U cells, an increase in osmolarity by 130mosm (OS) triggers cytoplasmic alkalinization. This is inhibited by DMA and enhanced by H_2DIDS. ION (1uM) elicits a slight cytoplasmic alkalinization which is also enhanced by H_2DIDS. Addition of DMA prior to addition of ION unmasks an acidification. PMA (100nM), however, does not elicit a change in pH_i regardless of whether or not DMA or H_2DIDS are present. In contrast, the N cell shows a slight alkalinization upon addition of PMA which is enhanced by H_2DIDS, and displays marked acidification if PMA is added in the presence of DMA. pH_i is also regulated by ION in the N cell. However, OS does not elicit a change in pH_i in the presence or absence of H_2DIDS or DMA. In contrast, the M cell is responsive to PMA, ION and OS. The data suggest that phorbol esters, intracellular calcium and osmotic shock exert their effects on pH_i through different mechanisms. (Supported by NIH grants AM 27495 and NRSA CA07924-01)

W-Pos291 CALCIUM UPTAKE AND INOSITOL TRISPHOSPHATE INDUCED CALCIUM RELEASE IN RAT BRAIN MICROSOMES. Jyotsna Shah and Harish C. Pant, Laboratory of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The present study attempts to characterize the calcium (Ca^{2+}) uptake and 1,4,5 inositol trisphosphate ($InsP_3$) induced calcium release in the isolated rat brain microsomes prepared by differential ultracentrifugation (J. Neuroscience, 5: 2609-2617, 1985). Electron microscopy showed that the microsomal fraction consisted of characteristic endoplasmic reticulum derived vesicular profiles and were free of mitochondria and plasma membrane contamination. Ca^{2+} uptake and release were determined by measuring the radioactive calcium trapped in the vesicles.

ATP dependent accumulation of Ca^{2+} was observed in the brain microsomes which was inhibited by Sodium Vanadate (100 uM) but remained unaffected by Ca^{2+} channel blockers e.g. Ruthenium Red, Verapamil or Nifedipine. Addition of A23187 showed a sudden large release of the sequestered Ca^{2+}. KCl provided the best environment for Ca^{2+} uptake which was reduced to 90% in NaCl medium (100 mM) and to about 45% in K-Glutamate (100 mM) or sucrose (250 mM) media. $InsP_3$ caused a rapid release of Ca^{2+} which was followed by a slow re-uptake. This release was found to be $InsP_3$ concentration dependent and was maximal at a concentration of 0.2 uM. $InsP_3$ induced Ca^{2+} release was also dependent on free extramicrosomal Ca^{2+} with maximal release at 5.0 uM. Ca^{2+} release induced by $InsP_3$ required the presence of potassium chloride (100 mM) in the medium, as no significant release was observed with $InsP_3$ in sucrose (250 mM) or sodium chloride (100 mM) media but sodium chloride itself released Ca^{2+} from these microsomes. These studies suggest that brain microsomes can be used to study the intracellular Ca^{2+} mibilization in neuronal system.

W-Pos292 FURTHER EVIDENCES FOR THE ENDOGENOUS ACTIVATOR AS A CYTOSOLIC REGULATOR OF GASTRIC H^+ TRANSPORT. Tushar K. Ray, Pratap K. Das and Sandip Bandopadhyay, Dept. of Surgery, SUNY Health Science Center, Syracuse, New York 13210.

An endogenous activator (AF) capable of stimulating the gastric H^+,K^+-ATPase activity has been purified to homogeneity from dog and pig gastric cells and found to be a dimer of two identical 40 kD_a subunits in the active state. Identical nature of the AF monomers was unfolded by the detection of lysine as the sole N-terminal amino acid. The AF from one species can stimulate the H^+,K^+-ATPase from another species and vice versa. Such cross-activation is consistent with the striking similarities in the amino acid composition between the two species,suggesting the existence of great degree of homology in the AF molecules from different species. Several unique features of the AF including the mechanistic aspects of AF activation, as well as interplay with second messengers like Ca^{+2},are revealed in the study. The AF appreciably enhances the affinity of the H^+,K^+-ATPase to K^+ (high affinity site) known to increase the turnover of the enzyme. To complement this K^+ affinity, the AF also enhances the ability of the H^+,K^+-ATPase to generate more transition state (E*.ATP) complex by increasing the entropy of activation ($\Delta S^{\ddagger}$) of the system as revealed from Arrhenius Plot of the temperature activation kinetics. In addition, the AF shows both positive cooperativity and strong inhibition depending on the concentration of the AF. Thus, up to the ratio of the H^+,K^+-ATPase and AF of about 1:2 (on the protein basis) the AF shows sigmoidal activation (Hill coefficient=4.5),but beyond such concentration a strong inhibition was observed. Finally, Ca^{+2} at low (2-4 μm) concentration strongly inhibits the AF stimulated H^+,K^+-ATPase. It is proposed that the AF may be acting as a link in the signal transducing cascade system between the intracellular second messenger and the final physiological response of gastric H^+ transport.

W-Pos293 G-PROTEIN DISTRIBUTION IN CARDIAC SL AND SR; COMPARISON TO SKELETAL MUSCLE SL AND SR.
Nancy Scherer*, María-José Toro¶, Charlotte Tate*, Mark Entman* and Lutz Birnbaumer¶.
Departments of Medicine (Cardiovascular Sciences) and Cell Biology¶, Baylor College of Medicine.

G proteins are obligate for receptor coupling to adenylyl cyclase and may also couple to guanylyl cyclase, phospholipase C and directly to ion channels. We mapped the distribution of G proteins in canine cardiac SR and SL, and in rabbit fast-twitch skeletal muscle SL, t-tubule, junctional SR and longitudinal SR. G proteins were identified by ADP-ribosylation with [^{32}P]NAD using cholera toxin (CTX) or pertussis toxin (PTX) and autoradiography following SDS-PAGE. For heart, but not skeletal muscle fractions, both quantitative and qualitative differences were found for labeling with PTX. Only quantitative differences were observed with CTX. PTX labeled 3 bands in cardiac SL but only 2 bands in all other fractions. CTX labeled 2 bands in all cardiac fractions tested, the lower band much more than the upper one. Quantitatively, labeling of cardiac SL by CTX was about 20-40 fold more than cardiac SR; with PTX, cardiac SL was labeled about 4 fold more than cardiac SR. In all skeletal fractions, PTX labeled 2 bands about equally and CTX labeled 2 bands also about equally. Labeling was most intense in the t-tubule fraction, followed by SL. Junctional and longitudinal SR labeled only minimally, thus G proteins in skeletal SR may be due to contaminating t-tubule.

In summary, cardiac SL and SR, skeletal SL and t-tubule, but possibly not SR, contain G_s, the CTX substrate, and the PTX substrates G_i and G_o. In addition, there is a 3rd PTX substrate which is unique to cardiac SL. The subcellular heterogeneity in G-proteins may reflect the distribution of second messenger systems and ion channels to which the G proteins are coupled. A functional role for G proteins in cardiac SR is suggested. Supported by Grants HL-13870, HL-31164 and Training Grant HL-07282.

W-Pos294 EGG JELLY INDUCES A FAST TRANSIENT HYPERPOLARIZATION IN *LYTECHINUS PICTUS* SEA URCHIN SPERM.
Marco González-Martínez and Alberto Darszon, Depts. of Pharmacology and Biochemistry, CINVESTAV-IPN, México City.

In many species sperm must undergo the acrosome reaction (AR) to fertilize eggs. In sea urchin sperm this reaction is induced by the egg jelly, the outer investment of the egg and involves ionic fluxes, the fusion of the acrosome vesicle with the plasma membrane and the formation of the acrosomal tubule. These events allow sperm to reach the egg plasma membrane and fuse with it. During the AR there are Na^+ and Ca^{2+} influxes and H^+ and K^+ efluxes, an increase in intracellular pH and a membrane potential depolarization. We have used the positively charged fluorescent dye, dipropylthiodicarbocyanine to follow the sea urchin sperm membrane potential changes induced by egg jelly with a time resolution of seconds. Previously we reported that jelly induces a depolarization (increase in fluorescence) probably associated with the influx of Ca^{2+}. This depolarization is preceeded by a fast quenching, which is partially seen even in the absence of sperm (García-Soto *et al.* Develop. Biol. in press). We have now found that part of the initial quenching observed in the presence of sperm is due to a transient hyperpolarization (~5 sec). This transient hyperpolarization is K^+ dependent and is inhibited at 25-35 mM KCl in sea water. The inhibition of the hyperpolarization parallels the inhibition of the depolarization and the AR. These results suggest that jelly induces a transient hyperpolarization mediated by an increase in K^+ permeability which may be involved in the triggering of the AR. Supported by CONACYT and the Ricardo Zebada Foundation.

W-Pos295 STRUCTURE AND THERMOTROPIC PROPERTIES OF MIXTURES OF ETHER-LINKED 1,2-DIHEXADECYL-SN-GLYCEROL-3-PHOSPHOCHOLINE (DHPC) AND ESTER-LINKED 1,2-DIPALMITOYL-SN-GLYCEROL-3-PHOSPHOCHOLINE (DPPC). J. T. Kim, J. Mattai and G.G. Shipley. Biophysics Institute, Boston University School of Medicine, Boston, Massachusetts 02118.

Mixed phospholipid systems of ether-linked DHPC and ester-linked DPPC have been studied by differential scanning calorimetry (DSC) and X-ray diffraction at maximum hydration (60 wt.% water). Pure DHPC shows three reversible endotherms: a main transition, Tm = 44.1°C, a pre-transition, Tp = 33.5°C, and sub-transition, Ts ∿ 4°C, while pure DPPC shows two reversible endotherms at 41.3°C (Tm) and 36.5°C (Tp). As DPPC is incorporated in DHPC bilayers over the range 0 - 100 mol%, Tm decreases progressively from 44.1°C in DHPC to ∿ 42°C over the range 10-90 mol% DPPC; Tp decreases more sharply, changing from 33.5°C in DHPC to 25.3°C at ∿ 42 mol% DPPC; further addition of DPPC increases Tp from 24.3°C at 59 mol% DPPC, to 36.5°C in pure DPPC. Ts remains constant at ∿ 4-5°C and is not observed above 20 mol% DPPC. At 23°C, X-ray diffraction of fully hydrated DHPC shows an untilted, interdigitated bilayer gel phase, d = 47.0Å (see also Ruocco et al. (1985) Biochemistry 24, 2406) into which ∿ 30 mol% DPPC can be incorporated. At 39 mol% DPPC a large increase in d is obtained (d = 65.6Å); with further addition of DPPC, d decreases slightly to 64.0Å in pure DPPC. Above 30 mol% DPPC, the interdigitated gel phase switches to a regular non-interdigitated bilayer gel phase. At 50°C, the liquid crystalline L_α phase is present at all mol% DPPC; d decreases slightly from 67.6Å for DHPC to 66.4Å for DPPC. Thus, at T > Tm, DHPC and DPPC are miscible in an L_α bilayer phase. In contrast, two different bilayer gel structures (interdigitated and non-interdigitated) are adopted depending on the DHPC:DPPC molar ratio.

W-Pos296 STRUCTURE OF POLYMERIZED DIACETYLENIC LIPID BILAYERS

David G. Rhodes[1], Steven Blechner[1], Paul Schoen[2], and Paul Yager[2]
(1) Biomolecular Structure Analysis Center, U. Conn. Health Center, Farmington, CT 06032 and
(2) Code 6190, Bio/Molecular Engineering Branch, Naval Research Laboratory, Washington, D.C. 20375.

Diacetylenic polymerizable lecithins have been shown to form multilamellar tubular structures (0.3-1.0 um diameter) upon cooling through their chain melting phase transition temperature (Yager et al. 1985; Biophys.J. 48, 899-906) or upon slow extraction from organic solvent. Several possible explanations have been offered for the spontaneous formation of these unusual structures. We have studied the structure of these membranes using low angle x-ray diffraction from partially dehydrated multilayers. The unit cell for these structures is unusually small(~60A) for the length of the hydrocarbon chain, suggesting tilting of the chains, interdigitation, or a combination of the two. We have carried out step-function electron density modeling calculations to help identify the components of the electron density profile. The mid-bilayer electron density and the positions of high electron density corresponding to the cross-linked region suggest that the lipids of opposing monolayers are interdigitated and that the chains are strongly tilted. A model structure consistent with all available data will be presented. We have also studied the temperature dependence of this structure in order to help explain the mechanism of tubule formation.
Supported by NIH (HL-07420 and HL-33026), the Whitaker Foundation, and the Patterson Trust Foundation and by the Defense Advanced Research Projects Administration.

W-Pos297 CORRELATION OF THE PHARMACOKINETIC PROPERTIES OF AMIODARONE, NIMODIPINE AND PROPRANOLOL WITH THEIR LOCATION IN A MEMBRANE BILAYER. M. Trumbore and L. Herbette, Univ. of Conn. Health Center, Biomolecular Structure Analysis Center, Farmington, CT 06032.

Amiodarone, nimodipine and propranolol are anti-arrhythmic agents with vastly different pharmacologic and pharmacokinetic properties. This study sought to provide a structural basis for these differences. Membrane partition coefficients of 10^6, $5x10^3$, and $2x10^2$, respectively, were determined for these agents. The removal of amiodarone that had been incorporated into purified skeletal muscle sarcoplasmic reticulum membranes was significantly more difficult compared to the removal of nimodipine and propranolol. X-ray diffraction provided the location of amiodarone in dipalmitoyl-phosphatidylcholine (DPPC) lipid bilayers. The average location of amiodarone was determined to be approximately 6A from the center (terminal methyl region) of the lipid bilayer. Previous structural studies of nimodipine in skeletal muscle sarcoplasmic reticulum showed that this drug is approximately 16A from the center of the bilayer, near to the hydrocarbon core/water interface, a location similar to that of propranolol in dimyristoyl-phosphatidylcholine (DMPC) lipid bilayers. These data indicate that when amiodarone partitions into a membrane bilayer, it is apparently too far removed from the hydrocarbon core/water interface (~10A) to be "washed out" in contrast to nimodipine and propranolol. The results presented here may explain the extreme persistence of amiodarone's clinical effects after its administration is terminated. This study may also provide one molecular criterion, namely, location in the membrane bilayer, which may dominate one facet of a drug's clinical half-life for activity. Supported by NIH HL-33026, Whitaker Foundation and the Patterson Trust Foundation. Dr. Herbette acknowledges his affiliation as an Established Investigator of the American Heart Association.

W-Pos298 DIFFUSIONAL DYNAMICS OF RHODAMINE B IN SARCOLEMMAL LIPID BILAYERS: IMPLICATIONS FOR DIHYDROPYRIDINE LATERAL DIFFUSION. Mason, R.P., Chester, D.W., and Herbette, L.G. Biomolecular Structure Analysis Center, Univ. of Conn. Health Center, Farmington, CT 06032.

1,4-dihydropyridine (DHP) calcium channel antagonists have potent clinical effects as direct inhibitors of voltage sensitive calcium channels in cardiac sarcolemma. A "membrane bilayer" pathway for DHP binding to the calcium channel has been proposed which involves 3 descrete steps; bilayer partition and orientation, lateral diffusion, and receptor binding. The lateral diffusion of an active DHP calcium channel antagonist, nisoldipine-rhodamine (Ns-R), was examined in cardiac sarcolemmal lipid multibilayers using the Fluorescence Redistribution After Photobleaching (FRAP) technique. The diffusion coefficient for this drug analog is the same as that for the phospholipid analog DiI at maximum bilayer hydration (3.8×10^{-8} cm^2/sec). We have examined the diffusional dynamics of free rhodamine (Rh) relative to the Ns-R complex and phospholipids (using both DiI and NBD-PE) to assess the relative contribution of the substituent fluorophore to the overall diffusion of the Ns-R complex. As a function of bilayer hydration, free rhodamine diffuses significantly faster than Ns-R and phospholipid analogs. These data suggest that rhodamine does not dominate the drug's diffusion within the bilayer but appears to diffuse independently within the aqueous region between bilayers. We are using X-ray diffraction to generate and model the CSL lipid bilayer profile structure to give rationale for the diffusional dynamics of Ns-R, DiI and free Rh. We are currently studying a DHP with intrinsic fluorescence for use in FRAP experiments to obtain an absolute diffusion coefficient for DHP in pure lipid and native CSL bilayers. Supported by research grants HL-07420, HL-33026, Connecticut Research Foundation, Whitaker Foundation and a gift from the Patterson Trust Foundation. Dr. Herbette is an Established Investigator of the AHA.

W-Pos299 THE MICROSCOPIC RATE CONSTANTS ASSOCIATED WITH DIHYDROPYRIDINE RECEPTOR BINDING: A FLUORESCENCE SPECTROSCOPY APPROACH. Chester, D.W. and Riordan, C.J., University of Connecticut Health Center, Farmington, CT. 06032

A membrane bilayer pathway has been proposed for the interaction of 1,4-dihydropyridine (DHP) calcium channel antagonists with receptors in cardiac sarcolemma. This model, involving drug partition and orientation within the bilayer followed by lateral diffusion and receptor binding is in contrast to a single step 'aqueous' binding pathway. We have shown that DHP have high partition coefficients and a time-averaged location near the hydrocarbon core/ water interface of the membrane bilayer. These data support the notion that bulk lipid partitioning is a first step in the overall receptor binding process suggesting that the membrane pathway is operable in this system. We are currently attempting to probe the kinetic events associated with this pathway using a fluorescence spectroscopy approach and correlate these data with equilibrium drug location. We have established that an active rhodamine 'tagged' DHP, nisoldipine-lissamine rhodamine (Ns-R), diffuses at a rate similar to that of phospholipid (3.8×10^{-8} cm^2/sec) in pure cardiac sarcolemmal lipid bilayers. Further, we have examined the fluorescence characteristics of several DHP's to define an analog with intrinsic fluorescence useful in the measurement of the partition and diffusion rate constants associated with the DHP receptor interaction. From these studies, a DHP has been identified with fluorescence that is sensitive to dielectric constant and spectra which may be suitable for fluorescence redistribution after photobleaching (FRAP) studies. We are currently persuing the use of this analog in rate studies to define the absolute values for the microscopic rate constants of bilayer partition and diffusion within the bilayer. This research is supported by NIH HL-33026, training grant HL-07420 and UConn Research Foundation.

W-Pos300 CHARACTERIZATION OF DISULFIDE POLYMERIZED PHOSPHATIDYLCHOLINES. T.M. Handel and J.D. Baldeschwieler. Chem. Dept., California Institute of Technology, Pasadena, CA 91125.

We are exploring the properties of polymerizeable thiol-containing PC lipids originally developed by Regen et al [JACS 107 42 (1985)]. In particular, the effect of the position of the thiol group and chain length (N) on macroscopic morphology, permeability, serum stability, and temperature dependent behavior are being examined.

EM, solute encapsulation and NMR results are indicative of vesicle formation by lipids with the thiols at C-2 of the fatty acyl chains (α-thiols) both before and after polymerization. In contrast, chain terminating thiols (T-thiol) form liposomes in the unpolymerized state (N=10,11) whereas Cu^{+2}/phenanthroline/H_2O_2 polymerization induces a reorganization into what appear to be "micelles". The temperature dependence of DPH polarization reveals a transition for the α-thiols which is unshifted upon polymerization and coincides with the temperature of maximal carboxyfluorescein release. Polarization detected transitions in the T-thiol (N=16), however, are shifted to higher temperature upon polymerization. These data illustrate the similarity of α-thiols and the uniqueness of T-thiols in comparison with nonpolymerizeable analogs, and may be correlated with the motional constraints imposed by polymerization. For the α-thiols, restricted motion is localized primarily to the aqueous interface as in conventional lipids, whereas for the T-thiols, it is introduced into the hydrocarbon region (^{13}C T_1 data). As our laboratory is concerned with the in vivo transport of chemotherapeutic agents, these studies should contribute to the rational design of liposomes and micelles as carriers of hydrophilic, hydrophobic, and covalently linked drugs. This work was supported by an NRS award (T32 GM07616) from NIH, a grant DAAG-29-83-K-0128 from ARO and gifts from Monsanto.

W-Pos301 A NOVEL SYNTHETIC GLYCOLIPID FOR ASSESSING LIPID-CARBOHYDRATE INTERACTIONS.
RAYMOND P. GOODRICH AND JOHN D. BALDESCHWIELER. CALIFORNIA INSTITUTE OF TECHNOLOGY, DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, NOYES LAB 127-72, PASADENA, CA 91125.

Carbohydrates added exogenously or incorporated into the bilayer in the form of glycolipids have demonstrated abilities to reduce vesicle leakage, preserve vesicles upon freezing and thawing, and stabilize H_{II} phase forming lipids in bilayers. We wish to report the synthesis and characterization of a class of glycolipids designed for the purposes of examining the nature and requirements for these interactions. The methodology employed consists of attachment of a carbohydrate moiety at a fixed, variable distance from the vesicle surface via a hydrophilic polymeric unit. This structure is incorporated into a vesicle by means of attachment to cholesterol at the 3 position.

Increasing amounts of this glycolipid in DPPC vesicles results in a lowering of the phase transition temperature from 42 to 35°C for a 2:1 lipid ratio as determined by DPH polarization. DPH polarization also reveals that microviscosity values for DOPC vesicles incorporating either cholesterol or the cholesterol derivative are of the same order of magnitude, indicating that the condensing effect of the cholesterol moiety is maintained. ^{31}P-NMR has been utilized to show that addition of 20 mole% of the cholesterol derivative to DOPE results in stabilization of this lipid in a bilayer structure at temperatures up to 45°C. This is corroborated with freeze fracture electron microscopy. The results presented here and the variability offered by the synthetic methodology permit future studies involving lipid polymorphism and vesicle stability in which the carbohydrate moiety may be utilized as a ligand in ligand-receptor interactions. This work was supported in part by a National Research Service Award (T32 GM07616) from the National Institute of General Medical Sciences, grant no. DAA6-29-83-K-0128 from the ARO, and a gift from Monsanto.

W-Pos302 CARBOHYDRATES AT THE CELLULAR SURFACE: STRUCTURE AND DYNAMICS VIA ^{2}H NMR. Ian C.P. Smith and Harold C. Jarrell, Div. Biol. Sci., National Research Council, Ottawa, Canada K1A 0R6.

The carbohydrates on the surfaces of cells are involved in a multitude of interactions with other cells, antibodies, hormones and drugs. The nature of these interactions is virtually unknown, and is usually inferred from the chemical nature of the compounds involved, or by studies of small molecules removed from the cells or synthesized as models. Deuterium NMR of labelled carbohydrate residues can reveal accurate detail about their organization and dynamics, even in a precipitated system. We have prepared a series of specifically deuterated glycolipids, and observed and analyzed their ^{2}H NMR spectra. The molecular order parameter, the molecular location of the axis of motional averaging, and the orientation of this axis with respect to the plane of the lipid bilayers (1,2) have been determined. The order parameters and locations of the ordering axes vary significantly from one carbohydrate framework to another. They are very sensitive to the nature of the glycosidic link. Conversely, in all systems studied to date the axis of motional averaging is found to be normal to the plane of the bilayer, probably due to axially symmetric motion of the entire molecule. The exocyclic moieties of the carbohydrate residues were found to manifest more than one rotamer in slow exchange on the ^{2}H NMR time scale (10^4 s^{-1}), usually not those found by X-ray diffraction studies of crystals.

The utility of this technique for the study of intact cells is now very obvious. We are presently applying it to membranes of A. laidlawii and H. cutirubrum.

1. I.C.P. Smith and H.C. Jarrell, Studia Biophysica 110, 163 (1985).
2. H.C. Jarrell, J.B. Giziewicz and I.C.P. Smith, Biochemistry 25, 3590 (1986).

W-Pos303 **EFFECT OF AMPHOTERICIN B AND FILIPIN ON MEMBRANE LIPID POLYMORPHISM.**
Jeffrey F. Ellena, Brian D. Hill, Glenn J. McGarvey and David S. Cafiso, Department of Chemistry and Biophysics Program, University of Virginia, Charlottesville, VA 22901.

^{31}P NMR spectroscopy and electron microscopy (EM, negative stain and freeze-fracture) were used to study the effect of the polyene macrolide antibiotics amphotericin B (AmB) and filipin on multilamellar membrane dispersions containing dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE) and ergosterol. Fully hydrated multilamellar dispersions consisting of 3:2 DOPE/DOPC and different concentrations of AmB and/or ergosterol were examined. The temperature at which an isotropic ^{31}P NMR spectral component appeared was reduced by ≈30°C or ≈75°C when 15% ergosterol or 15% AmB was present, respectively. At 30°C, the combination of 15% ergosterol and 15% amphotericin lead to a larger isotropic component than when either was present alone (30% ergosterol could not produce the same effect as the AmB ergosterol mixture). With 15% ergosterol DOPE/DOPC mixtures, substantial decreases in the temperature at which the isotropic phase appears is produced with AmB concentrations as small as ≈3%. EM results indicate that the appearance of the isotropic NMR components are not due to the formation of small bilayer vesicles, hence these components are likely associated with intermembrane attachment sites and/or "lipidic" particles. ^{31}P NMR results indicate that filipin disrupts bilayers more readily than does AmB. Further results on the dependence of membrane morphology on composition will be presented. Implications for the mode of action of polyene macrolide antibiotics will also be discussed.
[This research was supported by NIH grant GM35215.]

W-Pos304 INFRARED SPECTROSCOPY OF 2-[^{13}C]-DPPC BILAYERS: EFFECTS OF CHOLESTEROL, AMPHOTERICIN B AND CYCLOSPORINE A. P.M. Green, J.R. Mason, T.J. O'Leary, R.A. Dluhy and I.W. Levin, NIDDK, University of Virginia, Batelle Columbus Laboratory and FDA.

Vibrational spectra of the C=O stretching mode region reflect both conformation and motion of the phospholipid interface region. Interpretation is complicated by the overlap of contributions originating from *sn-1* and *sn-2* chains. To obviate this difficulty, we have synthesized DPPC in which the *sn-2* chain C=O, substituted with ^{13}C has a lower vibrational frequency. Anhydrous 2-[^{13}C]-DPPC has distinct spectral features at 1736, 1724, 1695 and 1681 cm^{-1}. When hydrated, the 1736 cm^{-1} feature shifts to 1738 cm^{-1} and the 1724 cm^{-1} shoulder disappears, while the lower frequency features coalesce into a single band with a peak frequency of 1691 cm^{-1}. On melting, these peaks shift to 1734 and 1694 cm^{-1} respectively, and broaden significantly. Addition of 20 mole percent cholesterol causes the *sn-2* chain C=O stretching mode to broaden significantly, without affecting the width of the *sn-1* chain feature. Addition of 50 mole percent cholesterol causes broadening of both features; the higher frequency *sn-1* feature is split, with distinct components at 1740 and 1732 cm^{-1} at all temperatures, suggesting coexistence of two phases. Thus changes in the lipid C=O vibrational spectrum are determined by changes in headgroup hydration and acyl chain packing. Hydration affects primarily the *sn-2* chain, and causes a change in rotational conformation around the glycerol C2-C3 bond. Addition of cholesterol, in contrast, selectively immobilizes the *sn-1* chain. These results will be compared with those for CSA and amphotericin B.

W-Pos305 MONITORING THE EFFECTS OF MODEL MEMBRANE PERTURBANTS BY SPATIALLY RESOLVING RAMAN SPECTRA ACROSS A CONCENTRATION GRADIENT. E. Neil Lewis and Ira W. Levin, Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892.

Alterations both in the relative order of the hydrocarbon chains (C-H stretching mode region) and headgroup hydration (C-N stretching mode region) of dipalmitoylphosphatidylcholine (DPPC) and di-O-hexadecylglycerophosphocholine (DHPC) bilayers were sensitively monitored as a function of temperature, ethanol-d_6 concentration and chloroform ($CHCl_3$) concentration using Raman spectroscopy.

Spectral data are obtained across a concentration gradient by spatially resolving the various domains within the multilamellar bilayers. Mole ratios of lipid/chloroform as high as 90:1 can be spectroscopically monitored, and the relative effects on either the headgroup or hydrocarbon chain region can be ascertained. Spectral changes are also presented as a function of temperature in order to clarify the disparate effects these three perturbants have on bilayer structure, as for example the induction of interdigitated and non-interdigitated phases in bilayers.

W-Pos306 BINDING KINETICS OF FLUORESCENT-LABELED PHOSPHATIDYLCHOLINE TO RAT LIVER NON-SPECIFIC LIPID TRANSFER PROTEIN. J. Wylie Nichols, Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322.

Phospholipid binding to rat liver non-specific lipid transfer protein (nsLTP) was demonstrated using a fluorescence dequenching technique. The fluorescence of 1-palmitoyl-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]phosphatidylcholine (P-C_{12}-NBD-PC) is highly self-quenched when contained as a high mole percent in phospholipid vesicles. These vesicles were used as donors for the insertion of P-C_{12}-NBD-PC into a non-quenching hydrophobic site on the nsLTP. Resonance energy transfer between P-C_{12}-NBD-PC and N-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine (N-Rh-PE) was used to study the kinetics of transfer of P-C_{12}-NBD-PC from P-C_{12}-NBD-PC:nsLTP complexes to vesicles containing N-Rh-PE and vice versa. Kinetic modeling of these data indicated that the transfer occurred both as a result of vesicle-nsLTP interaction and diffusion through the water phase. The P-C_{12}-NBD-PC dissociation rate constant from nsLTP to water (0.2 s^{-1} at 5°C) is four orders of magnitude faster than that from phospholipid vesicles to water (1.1 x 10^{-5} s^{-1} at 5°C), and the affinity constant for P-C_{12}-NBD-PC binding to a mole of phospholipid is 18 times that for a mole of nsLTP. These studies suggest a bind and release carrier model for the mechanism of nsLTP stimulated transfer of lipids between membranes. NsLTP collides with the membrane surface and binds a lipid molecule to a hydrophobic site. The resulting lipid:nsLTP complex dissociates from the membrane, rapidly equilibrates with the lipid in the water phase followed by rapid association of the free lipid and/or protein bound lipid with a membrane. (Supported by grants from NIH GM 32342 and American Heart Association, Georgia Affiliate.)

W-Pos307 DETERMINATION OF THE IONIZATION STATES OF NBD-LABELED LIPIDS IN MODEL MEMBRANES. Amitabha Chattopadhyay and Erwin London, Department of Biochemistry, State University of New York at Stony Brook, New York 11794-5215.

NBD (7-nitro-2-1,3-benzoxadiazol-4-yl)-labeled phospholipids are widely used as fluorescent lipid analogs in model membranes. The properties of NBD lipids will depend on their ionization state. The charge of the NBD group has been examined in model membrane vesicles over a wide range of pH (3-12) for NBD PE, in which the NBD moiety is attached to the headgroup phosphatidylethanolamine, and 12 NBD PC, in which the NBD group is attached to the 12th carbon atom in one of the fatty acyl chains of a phosphatidylcholine. An apparent ionization of the NBD group in 12 NBD PC and NBD PE with a midpoint of pH 11.5-12 is detected both by a decrease in fluorescence intensity and a blue shift in the wavelength of the absorbance maximum. This occurs both in small unilamellar (SUV) and large multilamellar vesicles (MLV). The changes are reversible. Analysis of zeta potentials obtained from electrophoresis measurements on MLV indicate that at neutral pH 12 NBD PC has no net charge whereas NBD PE has a negative charge. At high pH (greater than 11) 12 NBD PC also becomes negatively charged. We gratefully acknowledge the help of Stuart McLaughlin and Gerard Vaio in the electrophoresis measurements. This work was supported by N.I.H. grant GM 31986.

W-Pos308 SCHIFF BASES IN STRUCTURED MEDIA: A STUDY OF THEIR STABILITY WITH RESPECT TO HYDROLYSIS. P. Tancrède and C. Désilets, University of Quebec, Photobiophysics Research Center, Trois-Rivières (Quebec), Canada, G9A 5H7.

We have synthesized two Schiff bases from phosphatidylethanolamine (PE) and all-trans retinal (ATR), namely all-trans retinylidenedistearoyl-L-α-PE (ATR-PE(18:0)) and all-trans retinylidenedioleoyl-L-α-PE (ATR-PE(18:1)). The Schiff bases were put in two different structured media, i.e. in monolayer and in lipid vesicles, and their stability with respect to hydrolysis was studied as a function of the pH of the aqueous phase with which they were in contact. The range of pH values covered was from 3.0 to 9.0. When spread in monolayer, the surface pressure isotherm of the Schiff bases, in comparison to those of the pure components, allowed us to follow their stability at the interface. In bilayer, the particular spectral characteristics of the Schiff bases with respect to the pure ATR could tell us, on a semi-quantitative basis, if the Schiff bases were hydrolysed at a given pH. Our results have shown that ATR-PE(18:1) was resisting to hydrolysis in an aqueous medium, despite the fact that it results from a condensation reaction. In contrast, ATR-PE(18:0) did not show such a stability toward hydrolysis, thereby pointing to the role of the unsaturations in this phenomenon. Furthermore, we show that for ATR-PE(18:1) to be stable in an aqueous environment, its presence in a structured medium is mandatory.

W-Pos309 USE OF PYRENE AND 16-(1-PYRENYL)HEXADECANOIC ACID INCORPORATED IN UNILAMELLAR PHOSPHOLIPID VESICLES AS POLARITY PROBES OF THE HYDROCARBON CORE. G.P. L'Heureux and M. Fragata. Centre de recherche en photobiophysique, Université du Québec à Trois-Rivières, Québec, Canada.

The I/III ratio of monomer band emission of pyrene (PY) and 16-(1-pyrenyl)hexadecanoic acid (C_{16}PY) was used to investigate the polarity (dielectric constant, ε) of the hydrocarbon core of small, unilamellar phosphatidylcholine (PC) vesicles. First, we found that the I/III of PY and C_{16}PY is dependent on the mol % of incorporated probe in the lipid bilayers. However, a transition was observed around 1.0 mol %. Below this value the I/III ratio is constant: I/III=1.21 for PY and 2.80 for C_{16}PY. We also observed that above 1.0 mol % there is excimer formation. This may eventually obscur determinations of ε. We conclude thus that the use of PY and C_{16}PY as membrane polarity probes is restricted to concentrations < 1.0 mol %. The variation of the I/III ratio of PY is interpreted in terms of displacement of pyrene molecules into the neighborhood of the glyceryl moieties region of the PC vesicles at high mol % of the probe. A second conclusion is that the dielectric constant of the hydrocarbon core is comprised between 4 and 10. This can be explained either by penetration of H_2O molecules into the hydrocarbon region, or else by hydration of pyrene and the pyrenyl group of C_{16}PY. (This work was supported by grants from the N.S.E.R.C. Canada (A-6357), and the Fonds F.C.A.R. du Québec (EQ-3186)).

W-Pos310 ESR STUDIES OF THE EFFECT OF MELITTIN ON A PHOSPHOLIPID MODEL MEMBRANE. Timothy M. Phelps*, Timothy E. Kronenberg*, Stephen R. Wassall*, Marvin D. Kemple* and Franklyn G. Prendergast†, Department of Physics*, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223 and Department of Biochemistry and Molecular Biology†, Mayo Medical School, Rochester, MN 55901

Melittin, a major constituent of bee venom, is a peptide (molecular weight 3400) of 26 amino acid residues. It has a variety of effects on natural membranes, e.g. lysis and enhancement of phospholipase A activity, and on phospholipid model membranes, e.g. increased permeability and modified phase behaviour. We have employed ESR (electron spin resonance) of nitroxide spin labelled stearic acids intercalated into DOPC (dioleoylphosphatidylcholine) unilamellar vesicles to investigate the effect melittin has on acyl chain motion within the membrane.

The results show that melittin restricts molecular motion throughout the acyl chain, increasing order parameters S and correlation times τ_c in the upper and lower portions of the chain respectively. There is a discontinuity in slope of the dependence on melittin concentration at 5 mol%. The similarity in form of the concentration dependence recorded for vesicles in 20 mM and 1 M phosphate buffer (pH 7.5) is taken to support the contention that membrane bound melittin is monomeric.

W-Pos311 FLUORESCENT PROBES OF ELECTROSTATIC POTENTIAL 1 NM FROM THE SURFACE OF MEMBRANES. Anthony P. Winiski. Dept. of Biochemistry, SUNY, Stony Brook, NY.

The Gouy-Chapman (GC) theory describes adequately the relationship between the charge density and electrostatic potential at the surface of a bilayer membrane. It also describes the dependence of potential, $\Psi(x)$, on distance from the surface, x, at least for x > 2 nm, where the "hydration force" is negligible and measurements can be easily interpreted (Loosley-Millman, M.E. et al., (1982) Biophys. J. 40, 221-232; Mara, J., & Israelachvili, J.N. (1985) Biochemistry 24, 4608-4618). To study the electrostatic potential within the 0 - 2 nm region, I attached fluorescent probes to the sialic acid of the ganglioside G_{M1}. X-ray diffraction measurements demonstrate that the sialic acid of G_{M1} is 1 nm from the surface of a PC/G_{M1} bilayer (McDaniel, R.V., & McIntosh, T.J. (1986) Biophys. J. 49, 93-96). I incorporated these fluorescent gangliosides into neutral (PC) and charged (PS) phospholipid bilayers and quenched the fluorescence with the cations thallium and tempamine. The quenching is proportional to the concentration of these cations adjacent to the chromophore. I calculated the electrostatic potential at the chromophore by using the Boltzmann relation and obtained potentials of -39 and -35 mV for PS bilayers in 0.1 M $NaNO_3$ using anthraniloyl-G_{M1} and dansyl-G_{M1}. I also used thallium and tempamine to quench the fluorescence of chromophores located at the surface of the PS membranes: I calculated an average surface potential of -78 mV in 0.1 M $NaNO_3$, which agrees with the results of other experiments. The GC theory predicts the potential 1 nm from a membrane with a surface potential of -78 mV is -23 mV; this prediction agrees qualitatively with the experimental results obtained with fluorescent gangliosides. (Supported by NIH GM 24971 to Stuart McLaughlin).

W-Pos312 AN EXPERIMENTAL TEST OF THE DISCRETENESS-OF-CHARGE EFFECT IN POSITIVE AND NEGATIVE LIPID BILAYERS. Anthony Winiski, Alan McLaughlin†, Robert McDaniel, Moises Eisenberg and Stuart McLaughlin. Health Sciences Center, SUNY, Stony Brook, NY and †Department of Biochemistry & Biophysics, University of Pennsylvania, Philadelphia, PA.

The electrostatic properties of charged bilayers and the bilayer component of biological membranes are often described theoretically by assuming the charge is smeared uniformly over the surface. This is one of the fundamental assumptions in the Gouy-Chapman-Stern (GCS) theory. However, the average distance between the charged phospholipids in a typical biological membrane is 2-3 nm, which is 2-3 times the Debye length in a 0.1 M salt solution. Existing discreteness-of-charge theories predict significant deviations from the GCS theory for the adsorption of ions to such membranes. We considered the predictions of the simplest discreteness-of-charge theory (Nelson A.P. & McQuarrie, D.A. (1975) J. Theor. Biol. 55, 13-27), in which the charges are assumed to be fixed in a square lattice and the potential is described by the linearized Poisson-Boltzmann relation. This theory predicts deviations that are larger for counterions than for coions, and much larger for divalent than for monovalent counterions. We tested these predictions by measuring the adsorption of a fluorescent monovalent anion and a paramagnetic divalent cation to both positive and negative membranes, which we demonstrated experimentally had the same average surface potential. All our experimental results with probes, including those obtained on membranes in the gel rather than the liquid crystalline state, agreed with the predictions of the GCS rather than the discreteness-of-charge theory when the bilayers contained monovalent lipids. A simple calculation indicates that the agreement between the experimental results and the predictions of the GCS theory could be due to the finite size of the lipids. Significant deviations from the GCS theory were detected, however, when we used ^{31}P NMR to measure the adsorption of protons to the polyvalent lipid, phosphatidylinositol 4,5 bisphosphate. (Supported by NIH GM 24971 and Council for Tobacco Research grant 1493).

W-Pos313 **STOCHASTIC MODEL FOR ELECTRIC FIELD-INDUCED MEMBRANE PORES. MECHANICAL BREAKDOWN. REVERSIBLE- AND IRREVERSIBLE ELECTROPORATION OF STABLE AND METASTABLE MEMBRANES.**
Istvan P. Sugar, Department of Biochemistry. University of Virginia, Charlottesville, Virginia 22908 and Institute of Biophysics, Semmelweis Medical University, 1444 Budapest.

Electric impulses (1-20kV/cm, 1-5 μs) cause transient structural changes in biological membranes and lipid bilayers, leading to reversible pore formation (electroporation) with cross-membrane material flow and if two membranes are in contact, to irreversible membrane fusion (electrofusion). The stochastic process of electroporation is treated in terms of a general pore model. The number of lipid molecules in the pore wall is the stochastic variable of the model describing the pore size and stability. The exact solutions of the master equation reveal the different types of electroporation. When the electric field is applied to a metastable planar membrane the average pore size is very small at subcritical fields. After a longer time as a result of the pore size fluctuations one of the pores attains a critical size and the membrane becomes unstable (mechanical breakdown). But, upon applying a supercritical field, the membrane becomes unstable immediately. coherent opening of the pores takes place and the membrane ruptures (irreversible electroporation). If, however, the electric field is switched off, before the critical pore size is reached, the pores reseal completely (reversible electroporation). Applying the electric field to thermodynamically stable planar membranes the pore size is very small at subcritical fields (poreless phase). At supercritical fields the membrane remains stable, but becomes porous. The average pore size may be controlled by the electric field strength. This electric field-induced poreless $\rightleftharpoons$ porous phase transition is reversible.

W-Pos314 Manipulation Of Condensed Phase Lipid Domains In Electric Fields, A. Miller, W... Heckl, H. Möhwald, TU Munich, Physics-Dept. (E22 Biophysics), D 8046 Garching

Increasing the surface pressure above a critical value π_c the formation of condensed phase phospholipid domains in monolayers at the air/water interface can be observed by fluorescence microscopy. These domains are shown to exhibit a surface potential different from that of the fluid environment and therefore can be manipulated by applying inhomogeneous electric fields perpendicular to the water surface. The fields are applied by placing a small tip or ring electrode about 20 μm above the water surface and applying potentials up to $\pm$ 100 V.

It is shown that domains can be collected or repelled from the area under the electrode with the sign of the force as expected from the surface potential differences between fluid and condensed phase. Domains can be deformed, and restoring forces can be measured after switching off the applied field. This demonstrates the existence of elastic forces and in some cases the equilibrium nature of domain shapes.

The technique can be used to fix nuclei and observe domain growth under the electrode and to melt or to crystallize under the influence of electric fields. Work supported by the Deutsche Forschungsgemeinschaft.

Lit.: A. Miller, H. Möhwald, Europhys. Lett. 2 (1986) 67, J. Physique subm.

W-Pos315 DI- AND TRI-VALENT CATION-INDUCED PHOSPHOLIPID VESICLE AGGREGATION: EFFECT OF LIPID COMPOSITION ON ION BINDING SELECTIVITY SERIES. K.S. Leonards and C. Dhers, Dept. of Physiology and Cardiovascular Research Laboratory, UCLA School of Medicine, Los Angeles, CA 90024.

The aggregation behavior of PC/PE/PS vesicles was examined as a function of cation type and lipid composition. Vesicle aggregation was measured both as a function of [cation] and as a function of time at constant [cation]. The cations evaluated were La^{3+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Ba^{2+}, Ca^{2+}, Sr^{2+}, Mg^{2+}. The lipid composition of the phospholipid vesicles was systemically varied from PC/PE/PS (0/0/100 mole%) to (40/40/20 mole%) and (80/0/20 mole%). Our results indicate that the selectivity series for the ability of these cations to induce vesicle aggregation changed dramatically as a function of lipid composition, even though PS was the only anionic phospholipid present in all cases. These results suggest the possibility that variations in lipid composition among various cellular and subcellular membranes could lead to a marked and variable functional discrimination in cation/membrane interactions, even in a constant physiological milieu (i.e. multiple cations present). (Supported by Laubisch and Tuchbreiter endowments, NIH grant HL34517, and a AHA-GLAA Grant-in-Aid #843 G1-1.)

W-Pos316 EFFECTS OF POLYHYDROXYLATED SOLUTES ON THE PROTON AND POTASSIUM PERMEABILITIES OF LIPOSOMES. Gail L. Barchfeld[1] and David W. Deamer[2], Departments of [1]Animal Physiology and [2]Zoology, University of California, Davis, CA 95616

Liposomes are several orders of magnitude more permeable to protons than other monovalent cations, and the correlation of water and proton permeabilities has lead to the suggestion that protons may utilize hydrogen bonded chains of water molecules in hydrated defects, particularly at the membrane/water interface. If high proton permeability (P_{H^+}) results from intermolecular hydrogen bonds associated with the membrane, then alteration of these putative bonds, by the presence of solutes capable of forming hydrogen bonds with water molecules, should change P_{H^+} independently of potassium permeability (P_{K^+}). To test this, the P_{H^+} and P_{K^+} of liposomes prepared by reverse phase evaporation (egg phosphatidylcholine, egg phosphatidic acid, cholesterol, 55:20:25 mole ratio) were determined by monitoring the decay of transmembrane pH or potassium gradients in the presence of up to 0.1 M hydroxylated solutes in the membrane phase, based on their octanol/water partition coefficients. P_{K^+} increased with 1,3-propanediol, 1,2-propanediol, ethylene glycol, and 1,4-butanediol; and was reduced by glycerol and 1,2-butanediol. P_{H^+} increased with 1,3-propanediol and 1,4-butanediol; did not change with ethylene glycol; and was reduced by 1,2-propanediol, glycerol, and 1,2-butanediol. These results show that P_{H^+} may be altered independently of P_{K^+} and are consistent with the hydrogen bonded chain mechanism of transmembrane proton permeation. Supported by the Office of Naval Research contract N00014-85-K-0242.

W-Pos317 PHOTOVOLTAIC BEHAVIOR OF ELECTRON CONDUCTING BILAYER LIPID MEMBRANES (BLMs), Pawel Krysinski and H. Ti Tien, Membrane Biophysics Laboratory, Department of Physiology (Giltner Hall), Michigan State University, East Lansing, MI 48824

Modification of bilayer lipid membrane with TCNQ provides not only electron-conducting behavior of a membrane, but also endows it with light sensitivity, as studied by means of voltammetry and potentiostatic methods [see J. Electroanal. Chem., 174, 299, 1984; 211, 19, 1986]. In order to evaluate certain parameters of observed process e.g., photovoltage (E_{ph}), photoresistance (R_{ph}), dark resistance (R_m), the exchange and limiting currents, I_o and I_e, we propose the electrical equivalent circuit of a system. This, in dark conditions, consists of the impedance and voltage of redox reactions on either side of a membrane shorted by the membrane resistance R_m due to possible ionic leakage through the BLM. The current flow in this network was approximated by a Tafel equation modified by mass transfer effect. In light conditions, two additional elements were added, namely the photoresistance (R_{ph}) in parallel to R_m, due to the generation of additional charge carriers, and photovoltages (E_{ph}) of the same or opposite sign than E_{oc} (open circuit voltage). The system as a whole was then polarized from the external voltage source and the circuit response was fitted by means of computer simulation to match the experimental I/V data curves. This gives us the values of $R_m = 1 \times 10^7$ ohm cm^2, $E_{ph} = 15$ mV, $E_{oc} = 92$ mV ($E_{eq} = 118$ mV), $I_o = 1.51$ nA, $I_e = 128.7$ nA and charge transfer coefficient $n = 0.58$. Taking these as a starting point, one can evaluate more details about the mechanism of charge separation and transfer in such systems. [Supported by NIH grant GM-14971]

W-Pos318 DIELECTRIC PROPERTIES OF ADSORPTION SITES OF PENTACHLOROPHENOL IN LIPID MEMBRANES AND pK_a OF MEMBRANE-BOUND PENTACHLOROPHENOL. P. Smejtek, A. Barstad, and K. Hsu, Environmental Science and Resources Program, and Physics Department, Portland State University, Portland, Oregon 97207.

The results of our three experiments: (1) Solvatochromic shifts of UV absorption spectra of pentachlorophenol (PCP) in model solvents and adsorbed to membranes, (2) pK_a of PCP in bulk solvents and those adsorbed to membranes, and (3) pH dependence of membrane conductance, show how dielectric properties of the membrane/water interface determine membrane activity of the pesticide, pentachlorophenol. Values of local dielectric constant obtained from the cavity models of Onsager (1936), Block-Walker (1973), and Suppan (1983) are 8-9 for phosphatidylcholine (PC) and 17-20 for phosphatidylglycerol (PG) membrane. The apparent pK_a of PCP adsorbed to membrane at 0.1 M KCl were found to be 5.97 (PC), 5.75 (PC/CHOL = 70/30 mole fraction), 6.68 (PG), and 6.32 (PG/CHOL = 70/30). These values are different from the aqueous pK_a of 4.8. The intrinsic pK_a were evaluated to be 5.5-6.0 for PC and 5.2-5.4 for PG membranes. The dielectric constants obtained from the pK_a values are 10-22 for PC and 27-37 for PG membranes. The pH values at the maxima of PCP-induced membrane conductivity in PC and PG membranes are found to be correlated with the apparent pK_a values.

The results indicate that (a) even though PCP is located in the low dielectric environment, it interacts with the aqueous phase via hydrogen bonding, (b) adsorption/ionization sites of PCP in PG membranes are closer to the aqueous phase, and (c) cholesterol facilitates hydrogen bonding interaction between PCP and water, and facilitates PCP ionization in membranes.

Supported by NIH Grant ES 00937.

W-Pos319 STUDIES OF DOUBLE LAYER CAPACITANCE AT THE LIPID/WATER INTERFACE. Arnold D. Pickar, Julian Hobbs and Pavel Smejtek. Department of Physics and the Environmental Sciences and Resources Program, Portland State University, Portland, Oregon 97207.

We have measured the capacitance of individual lipid bilayer membranes as the ionic strength of the aqueous environment of each membrane is changed from that of de-ionized water to over 10^{-2}M NaCl. Preceding each measurement the membrane is stabilized at 20°C over a period of time. Glycerylmonoolein, glycerylmonoerucin, egg-PC/cholesterol and phophatidylglycerol/cholesterol membranes have been studied. Capacitances have been measured using three techniques: (1) using a computer to compare the phase of a sinusoidal membrane current (0.1 to 10 Hz) with that of a reference (resistive) current; (2) measuring total charge transfer to the membrane system by integrating the current flow following the application of a voltage step; and (3) observing the discontinuity in membrane current at the instant of voltage ramp reversal while applying a low frequency triangular waveform voltage. In all of these experiment no appreciable change in capacitance was observed as a function of ionic strength, contrary to expectations which assume the presence of diffuse double layers at the lipid/water interfaces. The result suggests the existence of mainly compact double layers or a substantial penetration of ions into the polar head group region upon application of an external voltage. These phenomena are being further explored in a series of complementary experiments involving the measurement of capacitance of thin, solid insulating films (e.g., SiO films) on which lipid monolayers are laid down. Supported by NIH Grant No. ES00937.

W-Pos320 CONDUCTANCE ROUTES FOR PROTONS ACROSS MEMBRANE BARRIERS

Mike Chang & John Bramhall. Microbiology & Immunology, UCLA Medical School, Los Angeles, CA 90024.

Simple phospholipid bilayers show a high level of permeability to protons; in spite of this fact, large proton gradients existing across such bilayers may decay very slowly. In sealed systems the free movement of protons across a membrane barrier is severely restricted by the coincident development of a proton diffusion potential. Using the fluorescent weak acid N-[5-(dimethylamino)naphthalenyl-1-sulfonyl]glycine (dansylglycine) to monitor the collapse of pH gradients across barrier membranes it is revealed that in strongly buffered systems movement of the small number of protons giving rise to this electrical potential is insufficient to perturb the proton concentration gradient; significant flux of protons (and hence significant collapse of the concentration gradient) can only occur if a) protons traverse the membrane as part of an electroneutral complex, or b) if there is a balancing flow of appropriate counterions. In weakly buffered systems, the small initial uncoupled electrogenic flux of protons may significantly alter the concentration gradient. This initial rapid gradient collapse caused by uncoupled electrogenic proton movements is then superimposed upon the residual collapse attributable to tightly coupled proton flux. The initial uncoupled electrogenic proton flux shows a temperature dependence very similar to that demonstrated for water permeation across simple lipid bilayers; upon cooling there is a sharp decrease in flux at the temperature coinciding with the main gel-liquid crystalline phase transition of the lipid.

W-Pos321 THE STRUCTURAL ORDER OF LANGMUIR-BLODGETT FILMS AS STUDIED BY FLUORESCENCE TECHNIQUES

Suzanne F. Scarlata, Hans Riegler, and Jane D. LeGrange AT&T - ERC, P.O. Box 900, Princeton, NJ 08540

Fluorescence emission, polarization and microscopy have been used to study structural properties of Langmuir-Blodgett films. Fatty acid films labelled with NBD stearic acid or pyrene dodecanoic acid were deposited on fused silica substrates in thicknesses ranging from 1 to 21 layers. Fatty acid order is observed to increase as the monolayer film at the air-water interface from which multilayers are deposited is compressed and maintained under higher surface pressures. The average orientational order and probe distribution were studied as a function of increasing number of layers.

Changes in fluorescence parameters with temperature were monitored in films deposited at different surface pressures and in films of varying number of layers.

W-Pos322 **THE SPIN LABEL 5-DOXYL STEARIC ACID IS *NOT* CONFINED TO THE PLASMA MEMBRANE OF TB AND CHO CELLS**

N.J.F. Dodd*, D. Nettleton, H.M. Swartz, and Philip D. Morse, II University of Illinois College of Medicine, Urbana, IL 61801 and *Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, England.

The doxyl stearic acid spin labels are highly lipophilic and readily partition into liposomes and cell membranes. Their exact distribution in multi-membrane systems such as eukaryotic cells has been a matter considerable controversy. We have recently carried out a series of experiments designed to resolve this problem for 5-doxyl stearic acid using spin-spin broadening to examine the extent of the lipid pools available to this spin label.

Measurements were made between 10^0Cand 40^0C on liposomes, human erythrocytes, TB cells, and CHO cells. TB and CHO cells were measured in gas-permeable Teflon tubing so that they remained oxygenated and the spin label would not be reduced. Corrections were made for differences in the diffusion constant of 5-doxyl stearic acid in the various membrane preparations and for the amount of spin label incorporated into the various preparations. We chose an increase of 10% in the mid-field line width as an indication of the onset of spin-spin broadening. The phospholipid content of the various preparations were quantified.

We observed spin-spin broadening of 5-doxyl stearic acid at probe to lipid rations of 1:50 in the liposomes, in erythrocytes at 1:25, and in TB and CHO cells at 1:20 for total phospholipid which corresponds to 1:1 for plasma membranes alone.

Our results clearly demonstrate that 5-doxyl stearic acid is not confined to the plasma membrane of TB and CHO cells but must also probe the internal membranes as well. Thus, in experiments using 5-doxyl stearic acid to study TB, CHO, and presumably other eukaryotic cells, less than 10% of the signal comes from the plasma membrane.

Supported by NIH grants RR 01811, GM 35534, CA 40665, and GM 34250.

W-Pos323 FLUORESCENCE LIFETIME AND PHOTOPHYSICAL CHARACTERISTICS OF THE MEMBRANE PROBE, 1-PALMITOYL-2-[[2-[4-(6-PHENYL-*TRANS*-1,3,5-HEXATRIENYL)PHENYL]ETHYL]CARBONYL]-3-*SN*-PHOSPHATIDYLCHOLINE (DPHpPC).

Barry R. Lentz, Stephen W. Burgess, Roberta A. Parente, and Enrico Gratton. Dept. of Biochem., University of North Carolina, Chapel Hill, NC 27514. Intr. by James R. White.

We have investigated the reason for the sensitivity of the fluorescence excited state lifetime of DPHpPC to the concentration of this probe in dipalmitoylphosphatidylcholine (DPPC) multilamellar membranes (Parente and Lentz, Biochemistry 24, 6178). First, multifrequency phase and modulation fluorescence data were fit adequately by a single exponential decay at high (500) lipid/probe ratio, but required a double exponential description at low (25) lipid/probe ratio. The main (and long) lifetime component (4.16 nsec, 20°C) at low lipid/probe (25) was significantly smaller than the single lifetime (7.11 nsec) observed at high lipid/probe (500), indicating an excited state complex at low lipid/probe ratio. The second lifetime resolved at low lipid/probe was quite short (1.00 nsec). Second, the fluorescence anisotropy of DPHpPC decreased from 0.165 (47°C) at 500 lipids per probe to 0.133 at 50 lipids per probe, suggestive of an altered polarity of the excited state or of excited state resonance energy transfer. The anisotropy was constant over the entire emission spectrum. Third, the ratio of fluorescence intensity to fluorescence lifetime decreased by a factor of 4.5 at low lipid/probe relative to high lipid/probe ratios, an observation inconsistent with simple collisional quenching. Finally, the excitation spectrum was only slightly altered and the emission spectrum was totally unaffected by variations in lipid/probe ratio. These data seem most consistent with a model involving an excited state complex that stabilizes the short-lived Bu* state of the DPH polyene.

W-Pos324 The Detection of Nonbilayer Lipid Phases using a Fluorescent Probe.

C.D.Stubbs, J.B.Hoek, T.F.Taraschi and E.Rubin. Department of Pathology, Thomas Jefferson University, Philadelphia PA19107

The changes in the fluorescence properties of n-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine (n-NBD-PE), incorporated into multilamellar phospholipid vesicles (MLV) at a concentration of 1mole%, were characterized during bilayer-nonbilayer transitions. The fluorescence intensity, anisotropy and lifetime(s) were all found to increase on adoption of the nonbilayer phase. There was also a marked blue shift in the fluorescence emission maxima. The NBD-group therefore appears to experience a more hydrophobic and restricted environment in nonbilayer phase lipids. Dibucaine also was able to trigger the fluorescence changes. When the bilayer stabilizers, polylysine or spermine were added, subsequent Ca^{2+} addition did not further effect the fluorescence of n-NBD-PE. The fluorescence changes were demonstable with cardiolipin (CL), DOPE/PS, CL/DOPC and mitochondrial phospholipids. The CL-Ca^{2+}interactions can be described in the following stages : $nCL(bilayer)+nCa^{2+}$-$n(CL\text{-}Ca^{2+})(bilayer)$=$(CL\text{-}Ca^{2+}{}_n)(lipidic\ particle)$=$(CL\text{-}Ca^{2+}{}_n)$ *(hexagonal)*. The fluorescence intensity increases for Ca^{2+} concentrations below 5mM were found to be stable after the initial increase, whereas for higher concentrations the CL precipitated due to the formation of the hexagonal phase. These findings strongly suggest that the lower Ca^{2+} concentration is triggering the formation of lipidic particles, step (2) above. The number of CL in a lipidic particle was calculated to 23 according to a cooperative binding model, in good agreement with the number which can be calculated from X-ray diffraction data, showing the usefulness of this type of approach.

W-Pos325 FLUORESCENCE AND EPR STUDIES OF THE $Ca(PS)_2$ PHASE. K. I. Florine and G. W. Feigenson, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

Steady-state fluorescence and EPR spectroscopy have been used to examine the nature of the Ca^{2+}-induced gel phase in phosphatidylserine (PS) multilamellar vesicles (MLVs) as well as the behavior of a variety of probe molecules in this phase. Anthroyloxy- and doxyl-labeled PS (12-AS-PS and (7,6)PS, respectively) exhibit greatly restricted and/or slow probe motion in $Ca(PS)_2$, even compared to thermotropic gel-phase lipid. In contrast, anthroyloxy- and doxyl-labeled phosphatidylcholine (PC), as well as fluorescent- and spin-labeled fatty acid derivatives, show no apparent change in probe motion in $Ca(PS)_2$ compared to fluid lamellar lipid. Doxyl-labeled phosphatidic acid, phosphatidylethanolamine, and phosphatidylglycerol show restricted motion in $Ca(PS)_2$ relative to fluid-phase lipid. The fluorescent probes diphenylhexatriene (DPH) and trans-parinaric acid methyl ester (tPnA-Me) show motional behavior in $Ca(PS)_2$ that is intermediate between that observed in fluid and in thermotropic gel-phase lipid.

In PS/PC MLVs, Ca^{2+} induces lipid phase separation into a $Ca(PS)_2$ gel phase and a fluid liquid-crystal PS/PC phase. The distribution of fluorophor probes between the two phases, expressed as a concentration ratio $R_{LC/G}$, is determined using fluorescence quenching with the result $R_{LC/G}$ = 100 in favor of the fluid phase obtained for 12-AS-PC, 18 for 12-AS-Me, 12 for DPH, 3 for tPnA-Me, and 1 for 12-AS-PS. EPR spectral simulations yield $R_{LC/G}$ = 0.45 for (7,6)PS. For M13 coat protein reconstituted into PS/PC MLVs, $R_{LC/G}$ = 25 in the presence of excess Ca^{2+}, indicating significant protein clearing from Ca^{2+}-induced gel phase regions in model membranes.

W-Pos326 CHOLESTEROL BEHAVIOR IN MODEL AND BIOLOGICAL MEMBRANES AS DETECTED BY CHOLESTATRIENOL FLUORESCENCE.
Philip L. Yeagle, Brian Crawford, Kathy Boesze, Arlene D. Albert and Chris Sprowl. Department of Biochemistry, SUNY/Buffalo School of Medicine, Buffalo, NY 14214.

Cholestatrienol and dehydroergosterol are two valuable, new fluorescent probes for cholesterol behavior. Cholestatrienol is the most similar to cholesterol in chemical structure. Cholestatrienol has been incorporated into pure DMPC multilayers. Steady state fluorescence anisotropy values are significantly greater if the lipid film for production of the DMPC multilayers is prepared from non-polar (anisotropy greater than 0.2) rather than polar organic solvents (less than 0.2). Similarly high anisotropy values (greater than 0.2) are obtained from the sterol probe in sonicated egg PC vesicles. Two biological membranes have been examined. In rabbit muscle sarcoplasmic reticulum, an anisotropy value greater than 0.2 is obtained when the probe is incorporated by incubation of the membranes with sonicated vesicles containing the probe. Cholestatrienol has been incorporated into cholesterol-depleted rod outer segment disks and an anisotropy value less than 0.2 is obtained. In the extracted disk membrane lipids, cholestatrienol exhibits an anisotropy significantly greater than 0.2. These data suggest that common model systems in which cholesterol has been studied may not accurately reflect cholesterol behavior in biological membranes.

W-Pos327 CORONENE: A PROBE FOR STRUCTURAL FLUCTUATIONS IN PHOSPHOLIPID BILAYERS.
*Lesley Davenport, #Jay R. Knutson and †Ludwig Brand, *Chemistry Department, Brooklyn College of CUNY, Brooklyn, NY 11210, #Laboratory of Technical Development, Bldg. 10, Rm. 5D-10, NIH, Bethesda, MD 20892 and †Biology Department, The Johns Hopkins University, Baltimore, MD 21218.

Fluorescence emission anisotropy is a well established tool for measuring order in lipid bilayers. Gel-to-liquid crystal transition (melting) curves derived from probes like DPH agree well with calorimetric experiments, but order parameters found on the microsecond timescale (e.g. by magnetic resonance) are often lower, implying further disordering. We previously reported (Davenport et al., (1983), Photochem. Photobiol., 37, S20) that coronene, with a mean lifetime greater than 200ns, was sensitive to order changes on this timescale. Further, its D_{6h} planar symmetry provides exclusive detection of out-of-plane rotations. In DMPC gel-state SUVs, correlation times (ϕ) on the order of 10^{-6}s are found, consistent with whole vesicle rotation. More importantly, a significant depolarization process is observed within a 10° band below the T_m requiring 20-200ns. In SUVs, artifacts due to 'radial strain' effects or lateral diffusion may occur. Time-resolved emission anisotropy studies have thus been focussed on the use of larger diameter vesicles and DPPC. LUVs were prepared by both ethanol injection and ether evaporation methods. In all cases, submicrosecond depolarizations were observed. This data suggests phospholipid packing fluctuations (or gel-fluid exchange) occuring on a 20-200ns timescale. Since coronene, like DPH, reorients rapidly in a fluid phase, the observed slow loss of anisotropy should selectively measure the gel-to-fluid rate. Applications using other long-lived probes and to systems with altered cooperativity will also be discussed. (†Supported by NIH Grant No. GM11632).

W-Pos328 FLUORESCENCE LIFETIME DISTRIBUTION OF PARINARIC ACID ISOMERS IN ISOTROPIC SOLVENTS. T. PARASASSI, G. DE STASIO, E. GRATTON and F. CONTI, Istituto di Medicina Sperimentale, CNR, Roma, Dipartimento di Chimica, Università La Sapienza, Roma and Dept. of Physics, University of Illinois at Urbana-Champaign.

The cis- and trans-parinaric acid decay has been studied in ethanol and in dodecane by multifrequency phase and modulation fluorometry over a wide temperature range. A triple exponential decay of the two fluorophores in both solvents has been already reported (Biochemistry 23, 1984, 5660), with the lifetime values and the associated preexponential factors being temperature dependent. Assuming a continuous lifetime distribution model for the analysis of the cis- and trans-parinaric acid fluorescence decay data,better fits have been obtained, with lower chisquare values than those obtained using a triple exponential model. Two distributional components were found for both isomers either in ethanol and in dodecane. In all cases the width, the center and the fractional intensities of the two distributional components were temperature dependent. The cis- and trans-parinaric acid decay behaviour has been attributed to different excited-state conformational energies of the fluorophores.

W-Pos329 **A FLUOROMETRY STUDY OF THE EFFECTS OF HYDROSTATIC PRESSURE ON THE LOCATION OF PRODAN IN LIPID BILAYERS AND CELLULAR MEMBRANES. Parkson Lee-Gau Chong, Dept. of Biochemistry, Meharry Medical College, Nashville, TN 37208 and Dept. of Biochemistry, Univ. of Illinois, Urbana, IL 61801.**

The effects of hydrostatic pressure on the location of 6-Propionyl-2-(dimethylamino)naphthalene (PRODAN) in phosphatidylcholine lipid bilayers and in goldfish brain synaptic membranes have been studied by fluorescence spectroscopy over the pressure range of 0.001 to 2 kbar. The emission spectrum of PRODAN in all the membrane systems examined exhibits two local maxima: one centers at 435nm; the other at 510nm. Based on the polarity studies of Weber and Farris (Biochemistry 18, 3075 (1979)), it seems that the 510nm peak arises from PRODAN in the polar region of the bilayer whereas the 435nm peak from PRODAN in the hydrophobic area. The intensity ratio of these two peaks, F_{435}/F_{510}, varies with pressure. In general, F_{435}/F_{510} increases as pressure increases; in the case of dimyristoylphosphatidylcholine multilayers DMPC(MLV), a dramatic change in F_{435}/F_{510} appears at the lipid phase transition pressure. In goldfish brain synaptic membranes, an isoemissive point at 490nm is observed. The changes in the emission spectrum are interpreted as a pressure-induced relocation of PRODAN from the "polar" site (the 510nm peak) to the "hydrophobic" site (the 435nm peak). That is, PRODAN favors a hydrophobic environment under pressure. In goldfish brain synaptic membranes, PRODAN polarization increases with pressure, giving dT/dP values of 15-20° kbar^{-1} for both sites. These values are comparable with those previously reported for lipid-involving processes, suggesting that the two sites are indeed lipid-associated (Supported by NIH grant GM 11223 to Prof. G. Weber).

W-Pos330 THE EFFECT OF POTENTIAL-SENSITIVE MOLECULAR PROBES ON THE NMR AND PHASE TRANSITION PROPERTIES OF PHOSPHOLIPID MODEL MEMBRANE PREPARATIONS, B.P. Bammel, J.A. Brand, D. Evans, J. Fumero, H. Hopkins, G. Pritchett, & J.C. Smith, Dept. Chemistry & LMBS, Georgia State U. Atlanta, GA

The effect of a number of potential-sensitive molecular probes of the polyene class on the NMR and phase transition properties of model membrane preparations formed from dimysteroylphosphatidyl choline (DMPC) doped in some cases with dimysteroylphosphatidyl glycerol (DMPG) has been investigated in an effort to develop a location model for these probes in biological membranes. The cyanines diS-C_3-(5) and diS-C_4-(5) caused the ^{31}P resonance from small unilamellar DMPC vesicles to be broadened with no change in the control vesicle chemical shift. The T1 and T2 relaxation times are reduced in proportion to the dye to lipid ratio. A number of negatively charged probes including several oxonols and merocyanine 540 (M540) are without effect on the DMPC vesicle ^{31}P NMR properties. As judged by electron microscopy investigations, the cyanine probe-mediated changes in the latter properties are due at least in part to an increase in vesicle size caused by an irreversible fusion process. Additional local perturbations of the phosphate group, however, cannot be eliminated. Calorimetric measurements on the pretransition and main chain melting transition in multilamellar DMPC preparations indicate that although all probes thus far employed broaden and reduce the cooperativity of these transitions, diS-C_3-(5) has the smallest perturbing effect when approximately the same quantity of each probe is bound to the lipid. These observations suggest that the cyanine occupies a primarily surface membrane binding site, screens surface charge, and promotes vesicle fusion. In mixed DMPC/DMPG vesicles, the effect of the cyanines is similar to that in DMPC only vesicles, but a decrease in the ^{31}P T2 value caused by M540 is observed indicating a local perturbation of the phosphate motion. Additional work on these model systems based on ^{13}C NMR is in progress. Support: NIH GM30552 and RR07171; NSF CDP-7924717, PCM-8200262, and DMB-8500319.

W-Pos331 FLUORESCENT DYES ENCAPSULATED IN LIPOSOMES: MECHANISMS OF THE FLUORESCENCE CHANGES. Raymond F. Chen and Jay R. Knutson, Lab. Tech. Development, NIH/NHLBI, Bethesda, MD 20892

Fluorescent dyes encapsulated in small unilamellar liposomes have been useful in the study of liposome properties, lysis, and fusion. A dye such as 6-carboxyfluorescein (6CF) is over 90% quenched when encapsulated at a concentration of 0.2 M, but the quenching is released upon lysis (Weinstein et al, Science 195, 489 (1977). The mechanism of the quenching is not altogether clear. Plant (Biophys. J. 49(2), 119a (1986) reported a shortening of the lifetime of xanthene dyes in proportion to the quenching, indicating a collisional deactivation mechanism. We have largely confirmed those results by noting a decrease in the lifetime of free 6CF upon raising the concentration from 10^{-6} M to 0.04 M; but when the encapsulated dye is examined, over 90% of the remaining fluorescence had a lifetime characteristic of the free dye (over 4 nsec). When the absorption spectra of free and encapsulated dye are compared, a large difference is noted. The appearance of a shoulder at 474 nm in the 6CF spectrum is reminiscent of dimer formation in concentrated fluorescein and other xanthenes noted by Förster et al. The predominant form of quenching in liposomes thus appears to be due to nonfluorescent dimer formation, but some dynamic quenching also occurs, possibly due to collisions with monomers or dimers. The spectral and lifetime changes noted for 6CF also occur for Sulforhodamine B. In the case of encapsulated pyrene tri- and tetrasulfonic acids, the high internal concentration results in excimer emission which is abolished upon lysis of the liposomes.

W-Pos332 THE DEVELOPMENT OF MEMBRANE POTENTIALS INDUCED BY EXTERNAL ELECTRIC FIELD PULSES CAN BE FOLLOWED WITH A POTENTIOMETRIC DYE. Zenobia Lojewska, Benjamin Ehrenberg, Daniel L. Farkas, and Leslie M. Loew, (Intr. by Ramadan I. Sha'afi), Department of Physiology, University of Connecticut Health Center, Farmington, CT 06032

The spatial variation in the membrane potential induced by an external electric field applied to an individual cell can be mapped with a fluorescent probe and digital video microfluorometry (Gross, Loew and Webb, 1986, Biophys. J., 50, 339-348). In this work we demonstrate that the time course of these induced potentials can also be followed. A model membrane system consisting of a hemispherical bilayer allowed convenient measurement of the dye absorbance change as a function of the bathing solution conductivity. The charging time of the membrane was inversely related to the aqueous conductance as predicted by the theoretical solution to Laplace's equation. The theory has also been extended to cases where the membrane conductance cannot be neglected. This condition can be explored experimentally via introduction of ionophores into the hemispherical bilayer membrane. Membrane conductance can be determined by conventional voltage clamp techniques on the same hemisphere for which the dye absorbance change records the time course for membrane charging by the external field. (Supported by USPHS Grant GM35063).

W-Pos333 DEHYDROERGOSTEROL FLUORESCENCE IN HUMAN SERUM LOW DENSITY LIPOPROTEINS. Greg Smutzer, Philip L. Yeagle, and Harvey A. Berman*, Dept. of Biochemistry, School of Medicine; and *Dept. of Biochemical Pharmacology, State University of New York at Buffalo, Buffalo, New York 14214.

Human serum low density lipoproteins (LDL) were labeled with the fluorescent sterol probe dehydroergosterol to detect cholesterol-rich regions in these macromolecular complexes. Steady-state quenching studies indicated that no more than approximately 10% of dehydroergosterol fluorescence was quenched by KI. These results suggested that the majority of dehydroergosterol fluorescence was either buried in the outer phospholipid monolayer or in the neutral lipid core of LDL. Small unilamellar vesicles (SUV) were used as a model of the outer phospholipid monolayer of LDL, and dehydroergosterol fluorescence in SUV was quenched approximately 50% by KI. Fluorescence lifetime studies of dehydroergosterol in LDL detected the presence of two lifetime components, a major component near 1 nsec. and a second minor component near 3 nsec. When fluorescence lifetimes were measured as a function of temperature from 18 - 35° C, no change in lifetime occurred near 30° C where the neutral lipid core of LDL undergoes a cholesteric phase transition. A small change in the relative amplitudes of these two lifetime components occurred in this temperature region. Time-resolved fluorescence anisotropy decays corrected for background fluorescence yielded two rotational correlation times for dehydroergosterol in LDL. At 24.9° C, these decays were 1.4 and approximately 92 nsec. Steady-state and time-resolved fluorescence measurements indicate that cholesterol distribution in LDL may be more complex than suggested by a two pool model for cholesterol distribution in LDL. P.L.Y. is a Research Career Development Awardee (HL-00937) and fluorescence lifetime instrumentation was made available by an NIH Regional Resource Grant (RR-01705-01).

W-Pos334 ALTERED RED BLOOD CELL MEMBRANE FLUIDITY IN RESPONSE TO ALUMINUM STRESS IS DETECTABLE WITH ESR AND THE POLARIZATION PROBE TMA-DPH. Christopher Weis* and Alfred Haug#. Departments of Physiology*, Microbiology# and Pesticide Research Center*,#, Michigan State University, East Lansing, MI 48824.

Aluminum toxicity is manifest in both plants and animals in a variety of forms. Inhibition of hexokinase, inhibition of the calcium regulatory protein calmodulin and aluminum binding to chromatin are all suggested as potential mechanisms of toxicity. Surprisingly little information exists concerning the membrane effects of this toxic ion or the mechanism of uptake of aluminum in cells.

Our investigations have shown that the titration of micromolar quantities of aluminum ions onto isolated red blood cell membranes causes an increase in the fluorescence intensity of the membrane probe TMA-DPH. Furthermore, fluorescence anisotropy of the probe increases significantly upon titration with aluminum ions. The addition of citrate prevents both an increase in fluorescent intensity and polarization. Electron spin resonance studies using the spin label 5-NS result in a decrease in the value of $2T_{//}$ upon titration with aluminum.

W-Pos335 **EQUILIBRIUM AND DYNAMIC LIPID STRUCTURE IN UNSATURATED ACYL CHAIN PHOSPHATIDYLCHOLINE-CHOLESTEROL-RHODOPSIN RECOMBINANT VESICLES AND ROS DISK MEMBRANES.** Martin Straume and Burton J. Litman, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.

Rod outer segment disk membranes and rhodopsin-containing, large, unilamellar Egg PC, DOPC (di-18:1-PC), and PAPC (16:0,20:4-PC) vesicles with and without cholesterol were examined between 5 and 37°C by phase-modulation fluorometry to characterize the effects of unsaturated acyl chain composition, cholesterol and rhodopsin content, and temperature on the lifetime and equilibrium and dynamic depolarization properties of DPH and TMA-DPH. Biexponential lifetime analysis indicated that rhodopsin increased the fraction of short lifetime fluorophores and reduced the individual population lifetimes as a result of energy transfer. Increasing cholesterol and reducing temperature did not alter the relative fractions of short and long lifetime fluorophores but did increase the lifetimes of both probes consistent with reduced water penetrability into the bilayers as a result of tighter lateral molecular packing. Disk membranes had probe lifetime properties most similar to cholesterol-containing vesicles. Probe depolarization, analyzed by an orthogonal, bimodal, Gaussian orientational distribution model, was hindered by increasing rhodopsin or cholesterol and by decreasing temperature for both probes. DPH equilibrium orientations were narrowed and redistributed parallel to the bilayer normal indicating less molecular disorder at the bilayer median. Bilayer interfacial and headgroup regions (probed by TMA-DPH) were also ordered but were less sensitive than the hydrophobic bilayer interior to changes in temperature or vesicle molecular composition. The equilibrium molecular order of disk membranes was similar to that observed in cholesterol-containing vesicles. Rates of probe depolarization were insensitive to rhodopsin content but were slightly accelerated by cholesterol. Rhodopsin and cholesterol both induced greater bilayer order but cholesterol increased the rate of molecular fluctuations whereas rhodopsin had no effect on lipid dynamics. Cholesterol and rhodopsin therefore each induce more ordered bilayer structure but only cholesterol reduces the cooperativity of lipid dynamics resulting in more limited but more rapid local lipid molecular fluctuations. Supported by NSF grant PCM-8316858 and NIH grant EY00548.

W-Pos336 EXCHANGE BEHAVIOR OF BILE ACIDS IN PHOSPHATIDYLCHOLINE VESICLES.
Donna J. Cabral, Donald M. Small and James A. Hamilton.
Biophysics Institute, Boston University School of Medicine, Boston, Massachusetts 02118.

The flip-flop of bile acids between monolayers of small unilamellar egg-phosphatidylcholine (PC) vesicles was determined by the temperature-dependent ^{13}C NMR spectroscopy. The resonances from the ^{13}C-enriched carboxyl carbon were used as probes of the bile acid environments in PC bilayers containing a second lipid component. Previous work showed that the rate constant for flip-flop of cholic (CA), deoxycholic (DCA) and chenodeoxycholic (CDCA) acids individually added to PC vesicles (~22:1 mol:mol, PC:bile acid) is dependent on the carboxyl ionization state and on both the number and position of hydroxyl groups (Biophys. J. 49:506a, 1986). This study examined the effect of (i) cholesterol and (ii) a second bile acid on the flip rate of bile acids in PC bilayers. The inclusion of small amounts of cholesterol (3-10 mol%) in the vesicles decreased the rate of transbilayer movement for DCA and CDCA slightly. For example, the rate constants for CDCA at 35°C were 100, 85 and 75 s^{-1} at 0, 5 and 10% cholesterol, respectively. In vesicles with 30 mol% cholesterol, no measurable flip-flop occurred for DCA and CA over 24h. These results suggest that the rate of bile acid transbilayer movement may be dependent on the bilayer fluidity. The rate constant for flip-flop of CA was also determined in the presence of an equal weight of DCA, glycocholate or methyl-cholate (~18:1 mol:mol, PC:total bile acid). For all three mixtures and at all temperatures examined (25-60°C), the rate constants were the same as those for CA alone. Thus, heterodimers of CA and DCA or conjugates of CA do not flip as a unit, implying that CA traverses the vesicle bilayer in monomeric form.

W-Pos337 PROTONS CAN CROSS THE LIPOSOMAL MEMBRANE ELECTRONEUTRALLY. Peter Nicholls, Dept. of Biol. Sciences, Brock University, St. Catharines, Ont. L2S 3A1, Canada.

Liposomes and proteoliposomes, as well as plasma membrane vesicles, if internally loaded with an indicator (phenol red or pyranine) show a biphasic internal pH change when the external pH is suddenly changed by addition of an aliquot of strong acid or base. The fast component of this change has been variously attributed to tightly bound externally facing indicator and to rapid electrogenic proton movement. This latter model requires a high membrane capacitance, whereas steady state ΔpH measurements during COV (cytochrome oxidase-containing vesicles) respiration indicate a rather low capacitance (<< 1μF cm^{-2}) in such systems. The fast H^+ movement phase is also sensitive to internal buffering and insensitive to ionophores, indicating that it is due to a limited but rapid electroneutral movement of an uncharged acidic or basic species in response to the initial pH gradient. Additions of small amounts of membrane permeable weak bases (the local anaesthetics butacaine, dibucaine and chlorpromazine) increase the magnitude of the fast phase; in the absence of ΔpH these amines induce transient internal alkalinizations. The postulation of a second (slow) type of electroneutral H^+ movement is needed to account for the simultaneous maintenance of ΔpH and Δψ in COV during respiration. Electrophoretic entry of cations into such COV (creating internal alkalinity) must be balanced by electroneutral cation exit (in exchange for protons). Unlike the fast H^+ movement, this relatively slow exchange may be catalysed by but not stoichiometric with the membrane components involved. Fatty acids may be implicated in both types of electroneutral proton movement. (Supported by Canadian NSERC grant no. A-0412).

W-Pos338 **SIMULATION OF LATERAL DIFFUSION OF PHOSPHOLIPIDS IN MEMBRANES.** W. E. Blumberg, Department of Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, N.J 07974

Computer simulation of lateral diffusion of the phospholipid building blocks of membranes has been carried out using a model of random positional interchange between nearest neighbors under a wide variety of assumptions and initial conditions. Both square and hexagonal lattices have been considered, and programs have been written for the VAX 11/785 which run in as little as 30 ms processor time per completed random walk, permitting "hands on" computation. The same program runs only five times slower on the AT&T PC 6300. Diffusion on square and hexagonal lattices show remarkably similar behavior, a result which does not permit one to say that one lattice symmetry is "better." Calculations have been made under conditions appropriate to both spin-exchange experiments using spin-labeled fatty acid probes and to excimer-formation experiments using pyrene-labeled hexadecanoic acid probes. The results show how both these experiments can easily be analyzed and interrelated. Both the jump frequencies and the diffusion coefficients can be interpreted simply from experimental results presented in a number of papers in the literature. In addition, lateral diffusion simulations have been carried out in the presence of different numbers of obstacles varying in size from 1 to 7 lattice spacings on a side. A mathematical model for lateral diffusion in the presence of such obstacles has been developed which adequately accounts for diffusional behavior in the presence of different sizes and shapes of obstacles as well as patterns produced by different rules governing possible overlap of obstacles.

W-Pos339 CONCENTRATION AND TIME DEPENDENT SELF-DIFFUSION OF INTERACTING MEMBRANE PROTEINS.

James R. Abney, Bethe A. Scalettar and John C. Owicki, Department of Biophysics & Medical Physics, University of California, and Division of Biology & Medicine, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Despite the high concentrations of intrinsic membrane proteins found *in vivo,* very little work has addressed the role protein-protein interactions play in modulating protein lateral diffusion. Beginning with an N-particle diffusion equation that describes a system of interacting particles, we have developed (following T. Ohtsuki (1982) *Physica 110A:* 606A) a formalism for determining a (time-dependent) self-diffusion coefficient for membrane proteins. The approach makes explicit use of the interprotein force and equilibrium distribution functions and contains no adjustable or phenomenological parameters. The information required in our calculations can be deduced completely from freeze-fracture electron micrographs that reveal protein positions (J. Braun, J.R. Abney and J.C. Owicki (1984) *Nature 310:* 316) or from Monte Carlo simulations of particle configurations. We have, therefore, the ability to study real physical systems and to analyze interaction potentials and protein densities that are not accessible experimentally.

We are addressing two important questions: (1) What is the effect of protein density on diffusion over distances that are many times the mean interparticle separation? The intermolecular potential and density of neighbors would certainly be expected to influence lateral motion that spans multiple interactions between particles. Longer-range diffusion underlies molecular motion on a cell-wide scale, and is the phenomenon studied in photobleaching recovery experiments. (2) How does short-ranged protein diffusion, when the particle is effectively between "collisions", differ from motion that is averaged over many interactions? Short-ranged motion bears most directly on cases where neighbor-neighbor interactions are important (e.g., diffusion-controlled reactions).

Preliminary numerical results suggest that at physiological densities long-ranged diffusion is retarded by a factor of two or more over the limit of infinite dilution. The short-ranged diffusion coefficient decays to the long-ranged value from a starting point near the dilute limit.

This work has been supported in part by NIH grant AI-19605. B. Scalettar was supported by J.E. Hearst and M.P. Klein through NIH grant GM30781 and DOE grant DE-AC03-763F0098.

W-Pos340 LATERAL DIFFUSION IN AN ARCHIPELAGO: THE DISTANCE DEPENDENCE OF THE DIFFUSION CONSTANT. Michael J. Saxton (Intro. by John C. Owicki), Plant Growth Laboratory, University of California, Davis, Calif. 95616 and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, Berkeley, California 94720.

Electron transfer in chloroplasts, mitochondria, and endoplasmic reticulum is thought to require lateral diffusion of mobile redox carriers. But the concentration of protein in these organelles is high, and according to percolation theory, long-distance diffusion is blocked when the concentration c of immobile obstacles is above the percolation threshold c_p. The question thus arises, what is long-distance diffusion? Or, when obstacles are present, how does the diffusion constant depend on the distance over which diffusion is measured? Monte Carlo calculations of diffusion on the triangular lattice give the mean square distance $\langle R^2(c,t)\rangle$ traveled by a particle in time t in the presence of an area fraction c of immobile obstacles. A normalized diffusion constant $D^*(c,R)$ can be defined as $d\langle R^2(c,t)\rangle/dt$. Monte Carlo results are shown in the figure. Here $D(c,0) = 1 - c$. For $c > c_p$, long-range diffusion is possible but with a reduced diffusion constant. For $c < c_p$, the particle is trapped on a finite cluster, so D goes to zero at a distance $R^2(c,\infty)$, proportional to the average cluster radius. (Supported in part by DOE contract AS03-80ER10700).