M-PM-Po47 QUERCETIN STIMULATION OF CA RELEASE FROM RABBIT SKELETAL MUSCLE SARCOPLASMIC RETICULUM. J. Watras, S. Glezen, C. Seifert, A. M. Katz (Intr. by R. L. Volle) Division of Cardiology, University of Connecticut Health Center, Farmington, CT 06032

To elucidate the mechanism by which quercetin enhances the rate of tension development in skinned muscle fibers (Shoshan PNAS 77: 4435, 1980), the ability of quercetin to stimulate Ca release from longitudinal tubule-derived SR (LSR) after phosphate-supported Ca uptake was examined at various external Ca^{2+} concentrations (Ca_o). LSR was isolated from rabbit skeletal muscle by zonal centrifugation, and Ca uptake measured at 25° C by Millipore filtration in 120 mM KCl, 5 mM MgATP, 50 mM phosphate, 40 mM histidine buffer (pH 6.8), 6 µg/ml LSR, and 10-75 µM $^{45}CaCl_2$. In all studies, 100 µM quercetin (which inhibits initial Ca uptake velocity 85%) was added at the time Ca content reached a maximum. At high Ca_o, where spontaneous Ca release followed initial Ca uptake (Katz, BBA 596: 94, 1980), addition of quercetin at the start of Ca release had little effect on release rate. At low Ca_o, where no spontaneous Ca release occurred, addition of quercetin after Ca uptake had no effect on Ca content. At moderate Ca_o, however, addition of quercetin after Ca content reached a maximum markedly stimulated Ca release after a 1-2 min. lag. Analysis of Ca release rate versus Ca_o at the time of quercetin addition demonstrated dramatic stimulation of Ca release over the pCa_o range 6.8-6.2. Analysis of unidirectional Ca fluxes at moderate Ca_o demonstrated that quercetin reduced Ca influx by 60%, whereas Ca efflux was increased 3-fold. Thus, the previously reported quercetin-induced increase in rate of tension development by skinned muscle fibers may result, at least in part, from sensitization of Ca-triggered Ca release to lower Ca_o. (Supported by NIH Grants HL-07420, HL-21812, HL-22135 and HL-26903).

M-PM-Po48 RAPID APPEARANCE OF CALCIUM SENSITIVITY IN THE ADP-INSENSITIVE PHOSPHOENZYME OF THE CALCIUM ATPase FROM SARCOPLASMIC RETICULUM. Jeffrey Froehlich and Phillip Heller, National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224.

The time course of dephosphorylation by ADP of the SR CaATPase phosphoenzyme can be resolved into rapid (ADP-sensitive) and slow (ADP-insensitive) components. We have previously (Fed. Proc. [1980] 39:2151) shown that the proportion of rapidly and slowly decaying phosphoenzymes does not change appreciably between the presteady state and steady state of phosphorylation indicating that ADP release and accumulation of the ADP-insensitive phosphoenzyme occur very rapidly. The ADP-insensitive phosphoenzyme observed at very short reaction times could be either E_1-PADP or E_2-P, the latter arising from the ADP-sensitive phosphoenzyme (E_1-PCa_2) by a conformation change and Ca^{2+} release. To determine which of these species is responsible for the ADP-insensitive component, we compared dephosphorylation of the phosphoenzyme by ADP in the presence and absence of a Ca^{2+} concentration jump. Since Ca^{2+} activates the back transition E_2-P → E_1-P at a low affinity site, an increase in the rate of decay of the ADP-insensitive component following a [Ca^{2+}] jump would indicate the presence of E_2-P. When 5 mM Ca^{2+} and 1.5 mM ADP were simultaneously added to leaky vesicles phosphorylated for 6 msec in a medium containing 10 µM ATP and 14 µM (free) Ca^{2+}, the ADP-insensitive component decayed at a rate of 170 s^{-1} as compared to 30 s^{-1} in the presence of ADP alone. The effect of Ca^{2+} added during dephosphorylation on the rate of disappearance of the ADP-insensitive phosphoenzyme was half-maximal at about 1 mM. Phosphate release occurring during the slow phase of dephosphorylation was less than stoichiometric with E-P decay. These results suggest that the ADP-insensitive component observed at very short reaction times is primarily E_2-P and that Ca^{2+} translocation and release take place very rapidly during ATP hydrolysis.

M-PM-Po49 REGULATION OF CARDIAC Ca^{2+}-Mg^{2+}-ATPase - A CONCERTED ROLE FOR CALMODULIN AND PHOSPHOLAMBAN, THE 22K REGULATORY PROTEIN IN Ca^{2+} TRANSPORT

ADIL E. SHAMOO AND INDU S. AMBUDKAR
Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Md. 21201

A Ca^{2+}-dependent calmodulin system has been proposed to be involved in the maintenance of the "normal" Ca^{2+} transporting activity of canine cardiac sarcoplasmic reticulum. This effect is mediated through phosphorylation of phospholamban, a 22K protein associated with this membrane. Activation of the Ca^{2+} transport ATPase is achieved by a c-AMP dependent phosphorylation (induced by β-adrenergic hormones) at a site distinct from the other. Removal of calmodulin from these membranes decreases Ca^{2+} transport by about 75%, but stimulates the c-AMP dependent phosphorylation by 30%. DOC treatment of calmodulin depleted membranes decreases the c-AMP dependent phosphorylation and the Ca^{2+} transport to basal levels and also removes the 22K protein from these membranes. Similar decrease in Ca^{2+} transport is observed on treating the membranes with phosphatase. Addition of both the c-AMP dependent system and the calmodulin dependent system restores Ca^{2+} uptake to 25% of the original control level.

This work is supported by the Office of Naval Research, Department of Energy, and the Muscular Dystrophy Association.

M-PM-Po50 UNIDIRECTIONAL CA FLUXES ACROSS THE SARCOPLASMIC RETICULUM MEMBRANE IN THE PRESENCE OF ACETYLPHOSPHATE. Hitoshi Takenaka, Priscilla Nash-Adler, and Arnold M. Katz, Department of Medicine University of Connecticut Health Center, Farmington, CT 06032

To define the mechanisms that mediate Ca efflux from sarcoplasmic reticulum vesicles (SR), unidirectional Ca fluxes in 0.1 mg/ml SR were studied at various acetylphosphate (AcP) and $CaCl_2$ concentrations in a medium containing 0.12 M KCl, 1 mM $MgCl_2$, and 40 mM histidine (pH 6.8) at 25° C. Ca content, measured by a Millipore filtration technique, reached a maximum approximately 4 minutes after reactions were started with concentrations of AcP less than 2 mM, regardless of the $CaCl_2$ concentration used. Spontaneous Ca release was not observed. Ca influx rates, measured at various times by pulse-labeling the medium of reactions containing non-radioactive ^{40}Ca with trace amounts of ^{45}Ca, were rapid in the initial phase of the reaction, but later slowed to a constant rate of approximately 30 nmol/mg·min. Ca efflux rates, calculated by subtracting Ca influx rate from the rate of change of total Ca content, were proportional to the Ca content (Ca efflux rate/Ca content= 0.33 min^{-1}) when Ca content exceeded approximately 30 nmol/mg. Initially during the filling of the vesicles with Ca, at Ca contents less than 30 nmol/mg, little Ca efflux was observed. Addition of 1 mM acetate had no effect on the Ca content, whereas EGTA and ADP, alone or together, induced a rapid Ca release. These data indicate that Ca efflux and net Ca release from Ca loaded SR can occur under conditions where reversal of the complete series of reactions responsible for Ca uptake (pump reversal) does not take place. (Supported by Grants HL-21812, HL-22135, and HL-26903 from the National Institutes of Health.)

M-PM-Po51 EFFECT OF CELL DENSITY ON HEXOSE TRANSPORT INTO L6 MUSCLE CELLS. Klip, A., Logan, W.J., and Li, G. Dep. Neurology, Research Institute, Hospital for Sick Children, Toronto.

Glucose transport in muscle is regulated by a variety of hormones, and by metabolic conditions inherent to this tissue. The structure of muscle tissue poses problems of accessibility and heterogeneity which complicate the determination of the molecular mechanisms underlying regulation of transport. We have shown that the myoblast L6 cell line is a suitable model system of muscle, and we have characterized kinetically and pharmacologically the uptake of 2-deoxy-D-glucose by these cells. The number of glucose carriers on the cell surface was determined by binding of the inhibitor Cytochalasin B (CB). (*Klip et al, Biochim. Biophys. Acta, 1981, in press*).

Thus it became possible to define whether changes in the number of glucose carriers are involved in regulation of transport. We have inquired whether L6 cells regulate hexose transport in response to variation in cell density. In monolayer cultures, the transport rate of hexose decreased when cells approached confluency. The decrease in transport rate was reflected both by an increase in Km and a decrease in Vmax. Cells were grown in monolayers at different densities, and then detached by trypsin. Differences in the rate of uptake of hexose (<0.5 mM) persisted in suspension, suggesting that a permanent change had occured. Current quantification of the number of CB binding sites will determine whether the change in Vmax is due to a decrease in the number of carriers.

Plasma membranes from L6 cells were prepared by cell disruption with N_2 cavitation, and purified in sucrose gradients. The membranes were identified by enzymatic determinations as well as ultrastructurally. Hexose transport studies in vesicles prepared from cells grown at different cell densities are underway, to define whether the change in transport rate is expressed in the isolated membranes. *Supported by MRC and MDAC (Canada).*

M-PM-Po52 THE EFFECT OF MERCURIALS ON CALCIUM RELEASE IN SARCOPLASMIC RETICULUM. Jonathan J. Abramson, Lyle Weden, Jonathan L. Trimm, Guy Salama*. Portland State University, Depts. of Physics and Chemistry, P.O. Box 751, Portland, OR 97207, and *Dept. of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

Mercury at micromolar concentrations is shown to cause an uncoupling of ATPase activity and active Ca^{2+} uptake in sarcoplasmic reticulum (SR) vesicles derived from fast skeletal muscle. Uncoupling is due to a dramatic increase in the permeability of the SR membrane to Ca^{2+}. This effect is irreversible. $HgCl_2$, CH_3HgCl and AgCl appear to bind to a sulfhydryl group on a protein that initiates the efflux of Ca^{2+} from SR vesicles. Measurements are made by passively loading SR vesicles with $^{45}CaCl_2$, and then diluting the vesicles into a Ca^{2+} free buffer. The Ca^{2+} remaining in the vesicles is measured as a function of time by millipore filtration techniques. In another set of experiments Ca^{2+} is actively taken up into SR vesicles upon the addition of ATP. Mercury is added, and Ca^{2+} efflux is examined as a function of time. Ca^{2+} efflux is measured either by following the movement of ^{45}Ca, or by spectrophotometric techniques using a Ca^{2+} indicator. External Ca^{2+} is monitored by the differential absorption changes of arsenazo III at 675-685 nm. The measurements show there to be fast Ca^{2+} efflux, initiated by the addition of Hg^{2+}, with a time constant less than two seconds.

Supported by the Medical Research Foundation of Oregon and the Health Research and Services Foundation of Western Pennsylvania.

M-PM-Po53 ACTIVE ENZYME SEDIMENTATION ANALYSIS OF SOLUBILIZED SARCOPLASMIC RETICULUM Ca^{2+}-ATPASE. Dwight W. Martin, Whitehead Medical Research Institute, Dept. of Physiology, Duke Univ. Med. Center, Durham, N.C. 27710.

The Ca^{2+}-ATPase of rabbit skeletal muscle sarcoplasmic reticulum can be solubilized in dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$) to yield a monomeric species. In the presence of substrates, this preparation has ATPase activity comparable to that of leaky sarcoplasmic reticulum vesicles. The monomeric state has been established by rigorous ultracentrifugal methods. Equilibrium ultracentrifugation analysis indicated that this preparation is greater than 85% monomeric. However, these studies were conducted under non-activating conditions. Therefore, the species actually responsible for the observed ATPase activity could not be directly determined. The possibility existed that under assay conditions, in the presence of a full compliment of substrates, the enzyme could oligomerize to form an ATPase active species. Accordingly, the monomer would possess only apparent activity. To test this possibility, active enzyme sedimentation analysis was performed on $C_{12}E_8$ solubilized monomeric Ca^{2+}-ATPase. The sedimentation coefficient of the active species was determined using a pH sensitive dye linked assay in a Vinograd Type I band forming analytical ultracentrifuge cell. Velocity sedimentation analysis under activating conditions yielded the same sedimentation coefficient ($s_{w,20} \simeq 5S$) as that observed for monomeric Ca^{2+}-ATPase under non-activating conditions. This value was invariant over a protein concentration range equal to or greater than that commonly employed in ATPase assay measurements. These data demonstrate that the monomeric form of Ca^{2+}-ATPase solubilized in $C_{12}E_8$ has full ATPase activity. (supported in part by NIH Fellowship 1 F 32 AM06579-01).

M-PM-Po54 CALCIUM BINDING AND ATPase ACTIVITY IN SOLUBILIZED SARCOPLASMIC RETICULUM. Jerson L. Silva* and S.Verjovski-Almeida, Department of Biochemistry, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro 21910, Brazil.

Passive calcium binding as well as calcium dependence of the ATPase activity were studied in purified sarcoplasmic reticulum ATPase and in ATPase solubilized by the nonionic detergent dodecyloctaethyleneglycol monoether ($C_{12}E_8$). Binding was measured by equilibrating the preparations with ^{45}Ca through chromatographic columns; the activities were preserved even after eluting the ATPase. Maximal activity was 3.5-4.5 μmol Pi mg^{-1} min^{-1} at 25° in both purified and solubilized preparations. The maximal amount of calcium bound to the ATPase was not changed upon solubilization. It was 9.0-9.1 nmol/mg, corresponding to one mol of calcium/mol ATPase polypeptide chains. Binding to the purified ATPase was cooperative with a Hill number of 1.96. After solubilization the cooperativity was lost, giving a Hill number of 0.93. The calcium dependence of the ATPase activity was cooperative in purified ATPase at all ATP concentrations tested. Upon solubilization the calcium dependence of the activity showed a cooperative and a non-cooperative component which were sensitive to the ATP concentration used in the micro to millimolar range.

Supported by grants from CNPq and FINEP (Brazil) and MDA (USA).

M-PM-Po55 INCORPORATION OF [^{32}P]PHOSPHATE INTO THE PHOSPHOLIPIDS OF DISEASED HUMAN MUSCLES. David L. VanderMeulen, Michael Bárány and Edward Abrahams, Depts. of Biol. Chem. and Orthopedic Surgery, University of Illinois, Medical Center, Chicago, IL 60612

The incorporation of [^{32}P]phosphate into phospholipids of human muscles and other tissues was determined by incubating freshly dissected tissues in physiological salt solution containing carrier-free $^{32}P_i$ at 37°C for 0.5-2.0 hr. The individual phospholipids were separated by thin-layer chromatography. The ^{32}P-incorporation was a linear function of the incubation time into the phospholipids. The rate of incorporation was expressed as nmol [^{32}P]phosphate incorporated per μmol phosphate in phospholipids, using the specific activity of [^{32}P]phosphocreatine, isolated from the same muscle, as a reference. The rate of incorporation was the highest in phosphatidylinositol compared to either phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, or sphinomyelin. The rate for various tissues differed greatly, over a range of more than two orders of magnitude. For example, 103 nmoles of [^{32}P]phosphate were incorporated per μmol of phosphatidylinositol in a spina bifida sample at 37°C per hr, but only 0.2 nmol [^{32}P]phosphate per μmol of phosphatidylinositol in human erythrocytes. In the case of spina bifida, the absolute rates of incorporation of [^{32}P]phosphate into all the phospholipids were at least an order of magnitude higher than those of normal muscle; also an unusually high percentage (14%) of the total phospholipid content for this patient was found to be lysolecithin, which can be a potent agent in cell lysis. For cases of facioscapulohumeral dystrophy, the relative rates of [^{32}P]phosphate incorporation for phosphatidylinositol, phosphatidylserine, and phosphatidic acid were consistently higher than normals, approximately 2, 10 and 5 times, respectively. (Supported by MDA).

M-PM-Po56 Ca MOVEMENTS IN FROG SKELETAL MUSCLE FOLLOWING A TETANUS. A.V. Somlyo, H. Gonzalez-Serratos*, H. Shuman, G. Mc Clellan and A.P. Somlyo. Pennsylvania Muscle Institute, University of Pennsylvania Medical School, Phila., PA and *Dept. of Biophysics, University of Maryland, Baltimore, MD

Bundles of approximately 20 semitendinosus fibers were frozen at 0.5 sec, 1.0 sec and 2.1 sec after a 1.2 sec tetanus at room temperature. The terminal cisternae (TC) in cryosections were analyzed by electron probe analysis with 50nm probes. Resting TC Ca (mmole/Kg dry wt.) was 117±48 S.D. (n=229) and decreased to 48±20 S.D. (n=222) with a 1.2 sec tetanus (1). The TC Ca increased to 79 (n=59), 87 (n=174) and 88 (n=109) mmole/Kg dry TC at 0.5, 1.0 and 2.1 sec following the tetanus.

The muscles had relaxed to baseline by 0.5 sec. after the end of the tetanus and by this time 31mmol/Kg dry TC had returned to the TC. Considering a TC volume of 4-5% of fiber volume and a cell H_2O content of 77%, the Ca uptake at the end of relaxation exceeded the amount of Ca that can be bound to the Ca-specific sites on troponin. The Mg content of the TC is increased during tetanus (1), was somewhat decreased at 2.1 sec (76mmol/kg dry TC), but did not reach resting values (59mmol/Kg dry TC) at 2.1 sec after cessation of stimulation. Experiments are currently in progress to determine in greater detail the post-tetanus time course of Ca, K and Mg movements.

1. Somlyo, A.V., H. Gonzalez-Serratos, H. Shuman, G. McClellan and A.P. Somlyo. J. Cell Biol. 90: 577, 1981.

This work was supported by HL15835 to the Pennsylvania Muscle Institute.

M-PM-Po57 INCORPORATION OF FREE FATTY ACID INTO SARCOPLASMIC RETICULUM. C. A. Napolitano, J. Watras, F. C. Messineo, A. M. Katz and L. Herbette. Division of Cardiology, University of Connecticut, Farmington, CT 06032

Electron microscopy was used to examine the association of added free fatty acids with sarcoplasmic reticulum (SR) membranes. Various concentrations (0-40 μM) of palmitate or oleate were added to SR vesicles (0-40 μg/ml) and centrifuged at 100,000 x g. Membrane pellets were fixed in tannic acid and osmium, and thin sections prepared according to standard procedures. Electron micrographs showed that increasing concentrations of oleate at a fixed SR concentration led to vesicle fusion and, at higher oleate concentrations, solubilization of SR vesicles. Palmitate did not cause vesicle fusion or solubilization, but instead caused the appearance of micelles. Although micellar formation with palmitate may have been due partly to centrifugation, the formation of palmitate-containing aggregates in solution is suggested by the ability of 40 μM palmitate to lower Ca^{2+} concentration measured optically with arsenazo III, a metallochromic Ca^{2+}-indicator. Freeze fracture micrographs of 40 μg/ml SR after addition of 40 μM palmitic acid demonstrated "lakes" (smooth regions) probably representing phospholipid and/or palmitate in the particle studded concave fracture plane. However, these smooth regions were absent at lower SR (20 μg/ml) and palmitate (20 μM) concentrations; instead there was an apparent increase in the separation of protein particles in the concave fracture plane. Preliminary data utilizing optical diffractometry of freeze fracture micrographs suggest that the lower palmitate concentration causes lateral separation of protein particles in the concave fracture face. Palmitate thus appears to be incorporated into the bilayer of the SR membrane. (Supported by NIH Grants HL-27630, HL-22135, HL-26903, HL-21812, HL-07420 and HL-00911).

M-PM-Po58 EVIDENCE FOR A LATTICE ORGANIZATION OF CALCIUM PUMP PROTEIN MOLECULES IN ISOLATED SARCOPLASMIC RETICULUM. L. Herbette[1], C.A.Napolitano[1] and P. Cooke[2]. Div. of Cardiology[1], Physiology Dept.[2], University of Connecticut, Farmington, CT 06032

Freeze fracture micrographs of isolated sarcoplasmic reticulum vesicles (SR) exhibited an ordered arrangement of protein particles in the plane of the membrane. Optical diffraction of freeze fracture micrographs was used to quantify this particle organization. High contrast masks were prepared to eliminate artefacts that could arise from particle distortions due to platinum shadowing. Approximately 50 vesicles were randomly selected from a broad field image and masks were prepared from relatively flat regions of each concave fracture plane to minimize distortions in the spatial distribution of particles due to vesicle curvature. A He-Ne gas laser light source was allowed to impinge at right angles to the plane of the high contrast mask, and diffraction patterns were recorded on Polaroid type 55 (positive/negative) film. Inspection of some of these diffraction patterns revealed a hexagonal lattice organization of the protein particles in the plane of the SR membrane with an interparticle separation of ~120 Å. Other patterns were characteristic of a square lattice distribution with random alterations in both the x and y directions, with x and y ~100 Å. The apparent transition in lattice structure may arise from the fluidity of the SR membrane, which could allow a shift of alternate rows of protein particles in the hexagonal lattice to degenerate to a square lattice, and vice versa. The apparent lattice organization in freeze fracture micrographs of SR affords the possibility of image reconstruction of particle structures that represent calcium pump protein monomers or oligomers. (Supported by NIH Grants HL-27630, HL-22135, HL-26903, HL-21812 and HL-07420).

M-PM-Po59 LIPID-PROTEIN INTERACTIONS IN SARCOPLASMIC RETICULUM. FLUORESCENCE STUDIES. Cecilia Hidalgo, Muscle Department, Boston Biomedical Research Inst., 20 Staniford St., Boston, MA 02114.

Addition of dansyl-PE (1-5% relative to SR lipids) results in immediate incorporation of 90-95% of the probe into the SR membrane. The membrane-bound probe establishes fluorescence energy transfer with tryptophan residues of the Ca^{2+}-ATPase enzyme. Changing $[Ca^{2+}]$ from pCa=8 to pCa=5, which produces a 5-10% increase in tryptophan fluorescence, does not modify the energy transfer efficiency, indicating that the tryptophan residues that respond to changes in $[Ca^{2+}]$ do not act as donors to dansyl PE. The efficiency of energy transfer decreases with increasing temperature; a change in temperature dependence was observed between 17-19°C. Fluorescamine-labeled intrinsic PE can also serve as acceptor, this obviates having to incorporate an external probe. In addition, the transfer process with fluorescamine-labeled PE requires closer approximation between donor and acceptor (R_o=2.4 nm) than with dansyl-PE (R_o=4.5 nm). The efficiency of energy transfer increases in two phases on increasing fluorescamine-labeled PE from 1 to 7 mol per mol of enzyme. Addition of SDS or deoxycholate revealed that for both acceptors energy transfer persists at concentrations of detergent that cause complete vesicular solubilization. A large excess of detergent has to be added to completeley abolish the energy transfer process, indicating that there is strong binding between the probes and the hydrophobic regions of the enzyme. Energy transfer efficiencies for purified ATPase preparations containing either dansyl-PE or fluorescamine-labeled PE are higher than for the native SR vesicles, suggesting that after purification the interactions between the enzyme and its surrounding lipids have been modified. (Supported by NIH grant HL23007).

M-PM-Po60 ISOLATION AND CHARACTERIZATION OF SARCOPLASMIC RETICULUM FROM MALIGNANT HYPERTHERMIC SWINE. C. Hidalgo, J. L. Fernandez, P. D. Allen, J. F. Ryan and F. A. Sreter. Dept. Muscle Research, Boston Biomedical Res. Inst., Dept. of Anesthesia, Harvard Medical School, Boston, MA

Malignant hyperthermia (MH) is a genetic disorder apparently affecting intracellular calcium movements. In both susceptible humans and swine it is characterized by a striking increase in metabolism in response to certain drugs and anesthetic agents (i.e. halothane). There are conflicting reports regarding the role of sarcoplasmic reticulum (SR) in MH. We have isolated SR vesicles from normal and MH susceptible (MHS) Poland China swine. The vesicular preparations from normal swine display protein and lipid compositions similar to those of highly purified rabbit skeletal SR. The uptake of calcium in the presence of oxalate and the phosphorylation of the Ca^{2+}-ATPase by ATP in normal swine SR are comparable to the values obtained with rabbit skeletal SR. All these properties indicate a high degree of purity in the swine SR preparation. Possible differences in the properties of normal and MHS SR are currently being investigated by measuring rate and maximal levels of phosphoenzyme formation, rate and maximum loading capacity of calcium transport, overall lipid content and composition, and fluidity of membrane lipids as probed by fatty acid spin labels. Preliminary results indicate that SR from the MHS swine has a decreased rate of calcium transport, lower steady-state phosphoenzyme levels and lower phospholipid content than normal swine SR. This would support the idea that a primary abnormality in MH resides in SR. Supported by NIH grant GM15904-14

M-PM-Po61 STIMULATION BY CALMODULIN OF $(Ca^{2+} + Mg^{2+})$-ACTIVATED ATPase ACTIVITY IN FAST SKELETAL MUSCLE SARCOPLASMIC RETICULUM VESICLES: POSSIBLE ROLE OF A PHOSPHOLAMBAN-LIKE PROTEIN.
M. A. Kirchberger and T. Antonetz, Dept. of Physiology and Biophysics, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. 10029, U.S.A.

We studied the effect of calmodulin (CaM) on $(Ca^{2+} + Mg^{2+})$-activated ATPase activity (Ca-ATPase) and phosphorylation of fast skeletal muscle (FS) sarcoplasmic reticulum (SR) vesicles. Ca-ATPase of microsomes prepared from rabbit adductor longus was measured in oxalate, [γ-^{32}P]ATP, EGTA or a Ca-EGTA buffer, and 6 µM CaM or cAMP plus cAMP-dependent protein kinase (cAMP-PK). $^{32}P_i$ formed was extracted as a phosphomolybdate complex into isobutanol. Ca-ATPase was calculated as the difference in rate of P_i liberation with EGTA or Ca^{2+}. Protein phosphorylation was determined by SDS-polyacrylamide slab gel electrophoresis followed by autoradiography. Ca-ATPase rates of ~0.3 and 0.5 µmol·mg^{-1}·min^{-1} at 0.33 and 0.66 µM Ca^{2+}, respectively, were increased 2 to 3 fold by CaM but were unaffected by cAMP-PK. Gels showed a M_r 22K protein to be labelled most prominently among other proteins with M_r ~90K, 62K, and 46K with CaM. With cAMP-PK, only the M_r 90K and 62K proteins were labelled to any significant extent. These data provide evidence for the existance in FS-SR of a M_r 22K protein, possibly phospholamban (PL), previously identified in cardiac (C) SR, where it appears to regulate Ca-transport. Unlike PL in C-SR and consistant with previous data from this laboratory, the M_r 22K protein of FS-SR is not phosphorylated by cAMP-PK, which also does not increase Ca-transport. However, like PL in C-SR, a M_r 22K protein in FS-SR is phosphorylated with CaM, which may mediate the effect on Ca-ATPase. (Grant-In-Aid, New York Heart Association; HL 15764, NIH)

M-PM-Po62 A FLUORESCENCE PROBE STUDY OF THE RAPID CALCIUM TRANSLOCATION REACTION IN SARCOPLASMIC RETICULUM. T.L. Scott and N. Ikemoto, Dept. of Muscle Res., Boston Biomed. Res. Inst. and Dept. of Neurology, Harvard Med. School, Boston, MA 02114.

The fluorescent thiol reagent N-(1-anilinonaphthyl-4) maleimide (ANM) has been used to label a specific sulfhydryl group of the Ca^{2+}-ATPase of sarcoplasmic reticulum (SR). In the presence of Ca^{2+}, addition of MgATP to ANM-labeled SR induces an increase of the fluorescence of the probe. This increase occurs only under conditions in which EP formation and Ca^{2+} translocation take place (Miki, Scott and Ikemoto, J. Biol. Chem. 256, 9382, 1981). We have attempted to specify the ATPase reaction step in which this fluorescence increase occurs. The time course of the fluorescence change has been measured by the stopped-flow technique and compared with those of EP formation and presteady-state calcium translocation determined by rapid chemical quench methods. The results indicate that the time course of the fluorescence change is slower than that of EP formation and parallels that of Ca^{2+} translocation. In further support of this conclusion, increasing [ATP] from 1 to 50 μM accelerates EP formation but not rapid Ca^{2+} translocation as determined by both probe fluorescence and quench techniques. The location of the ANM reporter appears to be in the A_2 tryptic subfragment of the ATPase. More precise localization of the labeled thiol within the primary structure of the peptide is in progress. (supported by grants from NIH and MDA).

M-PM-Po63 REVERSIBLE CONTROL OF OLIGOMERIC INTERACTION OF THE SARCOPLASMIC RETICULUM CALCIUM ATPASE WITH A CLEAVABLE CROSS-LINKING AGENT. N. Ikemoto and Y. Kurobe*, Dept. of Muscle Res., Boston Biomed. Res. Inst. and Dept. of Neurology, Harvard Med. Sch., Boston, Mass. 02114

The presence of mM ATP during the reaction of SR with the cleavable cross-linking reagent, dithio bis succimidyl propionate, maximally protects the enzyme against inactivation by chemical modification without affecting interpeptidic cross-linking by the cross-linker. We investigated the effect of $[Ca^{2+}]$ on cross-linking in the presence of ATP. Cross-linking at $pCa \simeq 5$ selectively inhibits the back reaction of the Ca^{2+}-controlled conformational changes, whereas cross-linking at $pCa \geq 8$ inhibits the forward reaction of these steps. Cross-linking at $pCa \geq 8$, but not at $pCa \simeq 5$, produces several additional changes: a) the rate of formation of one half of the EP becomes very slow, b) EP_{max} is reduced, and c) the second phase of the presteady state Ca^{2+} translocation, is selectively inhibited. All of these effects [except (c)] are fully reversed upon cleavage of the S-S bond of the cross-links, indicating that the effects are produced by cross-linking and not due to the chemical modification as such. These results suggest that cross-linking at $pCa \geq 8$ (viz. in the E-E state) reduces k_3, which in turn inhibits the subsequent EP formation and Ca^{2+} translocation reactions via $E^{\#}$. On the other hand, cross-linking at $pCa \simeq 5$ (viz. in the E^*Ca-$E^{\#}Ca$ state) selectively reduces k_{-2}. (Supported by grants from NIH and MDA)

$$\begin{matrix} E \\ E \end{matrix} \xleftrightarrow[Ca^{2+}]{1} \begin{matrix} ECa \\ E \end{matrix} \xleftrightarrow{2} \begin{matrix} E^*Ca \\ E \end{matrix} \xleftrightarrow{3} \begin{matrix} E^*Ca \\ E^{\#} \end{matrix} \xleftrightarrow[Ca^{2+}]{4} \begin{matrix} E^*Ca \\ E^{\#}Ca \end{matrix} \dashrightarrow \begin{matrix} \text{(EP formation and } Ca^{2+}\text{-} \\ \text{translocation)} \end{matrix}$$

(Ca^{2+}-controlled conformational change)

M-PM-Po64 EVIDENCE FOR AN ATP-SPECIFIC REGULATORY SITE IN DOG CARDIAC Ca^{2+}NTPase. C.A. Tate, T.D. Myers, R.J. Bick, W.B. Van Winkle and M.L. Entman. Section of Cardiovascular Sciences, Baylor College of Medicine, Houston, Texas 77030.

In dog cardiac sarcoplasmic reticulum (SR), GTP is an effective substrate for the Ca^{2+}NTPase enzyme in that it effects identical hydrolysis rates and has similar substrate affinity for the enzyme when compared to ATP. GTPase differs from ATPase in that it induces no acyl phosphate formation, is Ca^{2+} independent and allows no Ca^{2+} translocation. In the absence of Ca^{2+}, ATP competitively inhibits GTPase with a K_I (60-120 μM) equal to the K_M for ATP in the presence of Ca^{2+}. We reasoned that GTPase was an alternative enzyme cycle for the Ca^{2+}NTPase and that the enzyme mechanism was controlled by Ca^{2+} and nucleotide concentration. However, GTP in 100-fold excess over ATP did not inhibit ATP-dependent Ca^{2+} translocation or acyl phosphate formation. We postulated that, in the presence of Ca^{2+}, ATP is bound to a regulatory site which is ATP specific and dictates the classic Ca^{2+} translocation and acyl phosphate mechanism for which GTP is not a substrate or inhibitor. Compatible with the hypothesis is the observation that the nucleotide analog, AMP-P(NH)P, markedly inhibits (K_I=4-8 μM) the CSR GTPase in a non-competitive manner, whereas the more conventional GTP analog, GMP-P(NH)P, inhibits competitively with a K_I=40-80 μM. In contrast, in skeletal SR where ATP and GTP effects the classic Ca^{2+} dependent acyl phosphate-containing hydrolysis mechanism, both analogs are approximately equal inhibitors and are competitive. We suggest that, in CSR, AMP-P(NH)P acts at an ATP-specific regulatory site which regulates the classic ATPase mechanism. In skeletal SR, both nucleotides induce the classic enzyme mechanism and, presumably, nucleotide and their analogs occupy both the regulatory and catalytic sites. Supported by AHA (TX Affl.), HL 13870, HL 22856 and HL 17269.

M-PM-Po65 COMPETITIVE INTERACTIONS OF SODIUM AND CALCIUM DURING SODIUM-CALCIUM EXCHANGE IN CARDIAC SARCOLEMMAL VESICLES. John P. Reeves and John L. Sutko. Departments of Physiology and Internal Medicine, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The relationship between extracellular Na and Ca_2 levels and myocardial force development has frequently been described by the ratio $[Ca_o]/[Na_o]^2$, yet studies of Na-Ca exchange indicate a transport ratio of at least 3 Na per Ca. To resolve this apparent discrepancy, we have studied Ca uptake in sarcolemmal vesicles prepared from beef heart as a function of the intravesicular and extravesicular concentrations of Na. The initial velocity of Ca uptake exhibited K_m values for Na_i and Ca_o of 26-30 mM and 4-5 μM respectively. Consistent with previous demonstrations of the electrogenic nature of this transport process, a Hill coefficient of 2.3 was determined for the concentration dependence of Na_i. Na_o inhibited Ca uptake competively with a K_i of 10 mM. This inhibition was related to the square of the Na_o concentration and exhibited Hill coefficients which ranged between 1.4 - 1.8 in the presence of different levels of Ca_o. Thus, the data suggest that Na_i-dependent Ca uptake requires the participation of at least 3 internal Na ions per Ca but that only 2 external Na ions are necessary to inhibit this process. These findings are best explained by a carrier which has two distinct cation binding sites, a monovalent site which normally binds only Na and a divalent site which can bind either a single Ca or two Na ions. Such a carrier would be consistent with both the physiological and biochemical findings reported to date. Supported by NIH Grant No. HL-26810.

M-PM-Po66 PROTEIN AND LIPID ROTATIONAL MOBILITY IN SARCOPLASMIC RETICULUM OF DYSTROPHIC AND NORMAL CHICKENS. Diana J. Bigelow. Dept. of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455.

It has been suggested that alterations in the lipid environment of the Ca-Mg-ATPase of SR in chickens with genetic muscular dystrophy (line 413) correlate with its functional differences from that of normal chickens (line 412). Changes in lipid composition as well as increased lipid content have been reported for both human and animal genetic dystrophies. Consequently, rotational motion of both lipid and protein was measured by using spin labels and electron paramagnetic resonance spectroscopy (EPR): a short-chain maleimide spin label for sub-millisecond mobility of the calcium pump enzyme (using saturation transfer EPR) and the methyl ester of a long-chain fatty acid spin label to monitor fluidity (with conventional EPR). Ca-ATPase activity was enriched by active calcium phosphate loading, separation of loaded vesicles on a sucrose density gradient, and subsequent unloading with 10 mM MOPS and 100mM $MgCl_2$ at pH=6.0. Dystrophic SR showed similar protein mobility to that of normal SR and likewise there were no differences seen in lipid fluidity. The rotational motion of these samples correlated well with that seen in normal rabbit SR. In addition, phospholipid to protein ratios were slightly higher in dystrophic SR while its Ca-dependent ATPase activity and Ca-uptake rate was comparable to that of normal chicken SR.

M-PM-Po67 EFFECTS OF PARTIAL DELIPIDATION ON THE CA-ATPASE OF SARCOPLASMIC RETICULUM MEMBRANES. Thomas C. Squier. Department of Biochemistry; University of Minnesota Medical School; Minneapolis, Minnesota 55455.

Sarcoplasmic reticulum from rabbit skeletal muscle has proven a model membrane system for studying lipid-protein interactions within membranes. Electron paramagnetic resonance (EPR) is a valuable technique in elucidating these interactions (Thomas, Bigelow, Squier and Hidalgo; Biophys. J., 35, in press). A great deal of work has been done on the dynamics of the lipid component of the membrane using fatty acid spin labels, but it is equally important to study the physical state of the protein. The effect of the lipid/protein ratio (L/P) on the activity and the rotational correlation time ($1/\tau_c$) of the Ca-ATPase was measured. Changes in the lipid/protein ratio were accomplished by a series of partial delipidations using deoxycholate (DOC), and discontinuous sucrose gradients. The Ca-dependent ATPase activity of the control (no DOC) was virtually unchanged as a result of this treatment.

The Ca-dependent ATPase activity (measured in the presence of the ionophore A23187) increases with the mobility ($1/\tau_c$) of the enzyme. The activity varies in a sigmoidal fashion with the lipid/protein ratio, demonstrating a sharp decrease in activity below 40 lipids/protein. The protein mobility ($1/\tau_c$) varies in a linear manner with L/P, yielding complete immobilization ($\tau_c \geq 10^{-3}$ sec., as judged by EPR) at ~ 20 lipids/Ca-ATPase. Thus, there is a qualitative correlation between enzymatic activity and protein rotational mobility, but there is a difference in the quantitative dependence of these two parameters on L/P.

M-PM-Po68 35 GHz SATURATION TRANSFER EPR DETECTION OF SEGMENTAL MOTIONS IN THE SPECTRIN-ACTIN COMPLEX.

L. W.-M. Fung, Department of Chemistry, Wayne State University, Detroit, Michigan 48202 and M. E. Johnson, Department of Medicinal Chemistry, University of Illinois Medical Center, Chicago, IL 60680

The spectrin-actin complex from human erythrocyte membranes was extracted by low ionic strength buffer, and labelled by an N-ethyl maleimide-analogue nitroxide spin label for electron paramagnetic resonance (EPR) studies. Conventional EPR detection at 9 GHz (X-band) microwave frequency shows relatively immobilized, mixed, multicomponent motions, which are difficult to analyze. Saturation transfer (ST) EPR detection at 35 GHz (Q-band) frequency provides improved spectral resolution and motional sensitivity for the spectrin-actin complex. Computer spectral subtraction was used to analyze the Q-band ST-EPR spectra and revealed that there are at least three types of segmental or fluctuational motions. These motions of the complex are classified as fast, slow and very slow motions. The fast motion (faster than 10^{-9} sec in rotational correlation time) is a minor component and is very sensitive to sample conditions including temperature and pH. The slow motion is also temperature and pH dependent (the apparent rotational correlation times were 10^{-4} - 10^{-6} sec) whereas the very slow motion appears to be insensitive to pH and temperature changes (the apparent rotation correlation times were slower than 10^{-3} sec).

(Supported in part by grants from the Michigan Heart Association and the NIH. LWMF is an NIH Research Career Development awardee. MEJ is an Established Investigator of the American Heart Association).

M-PM-Po69 INHIBITION OF ERYTHROCYTE MEMBRANE-ASSOCIATED PROTEIN KINASE BY SUBSTITUTED PHENOTHIAZINES.

Mark Ros and Victor Glushko. Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140.

Substituted phenothiazines perturb both membrane structure and function. The activity of membrane-associated protein kinase from human erythrocyte ghosts was investigated with respect to inhibition by chlorpromazine, thioridazine, trifluoperazine, and unsubstituted phenothiazine. Hemoglobin-free erythrocyte membranes were preincubated with phenothiazines and subsequently assayed for protein kinase activity using [γ-^{32}P]ATP, membrane proteins and protamine as substrates. Membrane protein phosphorylation was more sensitive to phenothiazine inhibition than the phosphorylation of protamine. Preincubation with substituted or unsubstituted phenothiazines (0.3 mM) resulted in a significant decrease in the apparent V_{max} and in the apparent K_m for ATP and protamine. Higher concentrations (1.0 mM) of phenothiazines caused a further decrease in these kinetic parameters. The noncompetitive inhibition of membrane-associated protein kinase by phenothiazines potentially resulted from a change in membrane structure, which affected both the catalytic and substrate binding properties of the enzyme. The inhibition of protein kinase activity by 1 μM to 1 mM chlorpromazine, thioridazine, and trifluoperazine was unaffected by 154 mM NaCl. In the presence of salt, protein kinase activity was more susceptible to inhibition by substituted phenothiazines in the milimolar range. The NaCl effect and the observation that a progressive decrease in enzymatic activity parallels the increase in phenothiazine-to-membrane ratio, strongly suggest a partitioning process. The inhibition of protein kinase activity by phenothiazines could involve a nonspecific mechanism due to perturbation of membrane organization.

M-PM-Po70 THE EFFECT OF THIORIDAZINE ON BASAL AND cAMP-STIMULATED ACTIVITY OF MEMBRANE-ASSOCIATED PROTEIN KINASE IN HUMAN ERYTHROCYTES.

Mark Ros and Victor Glushko. Department of Biochemistry, Temple U. School of Medicine, Philadelphia, PA 19140.

The effects of thioridazine on erythrocyte membrane protein kinase were studied as a function of phenothiazine concentration in the presence and absence of adenosine-3',5'-monophosphate (cAMP). Samples of hemoglobin-free erythrocyte ghosts were preincubated at 37°C with thioridazine and subsequently assayed for protein kinase activity using [γ-^{32}P]ATP and protamine as exogenous substrates. The cAMP-stimulated phosphorylation of protamine was inhibited by as low as 3 μM thioridazine, while both basal protamine phosphorylation and membrane protein phosphorylation remained essentially unchanged. Higher concentrations of thioridazine (0.3 to 1.0 mM) did not produce a significant change in any of the phosphorylating activities. The cAMP activation index, defined as the ratio of protamine phosphorylation in the presence or absence of cAMP, was used to describe the cAMP effect. In the presence of 3 to 100 μM thioridazine, the cAMP activation index showed a progressive decrease from its maximal value to 1. There was no significant change in this parameter at 100 μM or higher thioridazine concentration. The effect of thioridazine on cAMP stimulation of membrane-associated protein kinase activity occured at a much lower concentration than that required for the total inhibition of the basal phosphorylating activity. The stimulation of protein kinase activity by cAMP was much more sensitive to inhibition by thioridazine and other substituted phenothiazines. The results suggest that different concentrations of phenothiazines induce at least two distinct effects on membrane organization and appear to affect different membrane functions.

M-PM-Po71 CALMODULIN-DEPENDENT SPECTRIN KINASE ACTIVITY IN RESEALED HUMAN ERYTHROCYTE GHOSTS. M.J. Nelson and W.H. Huestis. Department of Chemistry, Stanford University, Stanford, California 94305

Membrane protein phosphorylation has been studied in resealed human erythrocyte ghosts by measuring the incorporation of phosphorus-32 into spectrin and band 3. Norepinephrine- and calcium-stimulated phosphate incorporation was diminished in ghosts depleted of calmodulin. Ghosts prepared with endogenous calmodulin showed calcium- and norepinephrine-stimulated protein phosphorylation only when the ghosts had been resealed in the presence of [γ-^{32}P]ATP. Ghosts resealed with or without calmodulin in the presence of unlabeled ATP showed no net gain or loss of ^{32}P when exposed to norepinephrine or a calcium-specific ionophore. These observations suggest that calcium and norepinephrine stimulation of membrane protein phosphorylation is mediated by calmodulin-dependent spectrin kinase activity, and not by increased turnover of spectrin ATPase or by inhibition of phosphospectrin phosphatase.

M-PM-Po72 **MEMBRANE ASSOCIATION OF A PHOSPHOPROTEIN PHOSPHATASE FROM THE HUMAN ERYTHROCYTE.** **Dennis Carroll, Peter A. Kiener, Paul vom Eigen and Edward W. Westhead. Univ. of Massachusetts, Amherst, MA 01003.**

It has previously been reported that the human red blood cell possesses both a cytosolic and a membrane-bound phosphoprotein phosphatase but the relationship between the two enzymes, or forms of enzyme, is not clear. We have previously isolated and partially purified, at 4°C, the cytosolic enzyme. This phosphatase hydrolyses phosphospectrin, phosphocasein and phosphorylated pyruvate kinase; activity against all substrates is stimulated about 10-fold by 0.1-1mM Mn^{2+}. We have now found that there is reversible temperature-dependent association between the enzyme and the erythrocyte membrane. The binding which is favored by higher temperatures occurs within the intact cell and between enzyme and broken membranes. Furthermore, activity distribution studies show that unknown activators or inhibitors are released from the enzyme under conditions which alter the attachment of the enzyme to the membrane. If cells are maintained at 37°C during washing and lysis the enzymatic activity, against phosphocasein, associated with the complete lysate, the supernatant of the lysate (centrifuged at 12,000 rpm for 20 minutes) and the membrane pellet are in the ratios of 1.0:1.24:1.4 respectively. The same fractions prepared at 4°C have activity ratios of 1.0:1.6:0.70 respectively. If red cells stored at 4°C are incubated at 37°C before lysis the activity ratio of the supernatant to membrane pellet changes from 1.0:0.95 at 4°C to 0.38:1.0 at 37°C. Extraction of membrane proteins with 1 M KCl results in the release of much higher amounts of phosphatase activity than seen in the complete lysate. Clearly, the red cell membrane plays a very important role in controlling the activity of the phosphoprotein phosphatase. **(Supported by NIH Grant GM 14945)**

M-PM-Po73 ENVIRONMENTS OF TYROSINE AND TRYPTOPHAN OF RHODOPSIN IN BOVINE DISK MEMBRANES AND COMPARISON WITH BACTERIORHODOPSIN. U.P.Andley and B. Chakrabarti, Eye Research Inst., Boston, MA. 02114

The UV emission spectrum of rhodopsin in bovine disk membranes is characterized by emission from both tyrosine and tryptophan residues. The emission maximum for tryptophan shifts from 325 nm to 330 nm as excitation wave length is varied from 275 to 295 nm and a band at 335 nm appears upon excitation between 300-305 nm, suggesting the presence of tryptophan residues in different environments in the protein molecule. At pH>10, ionization of tyrosine to tyrosinate is suggested by a shoulder at 340 nm. However, no shift in the emission maximum is observed indicating that unlike bacteriorhodopsin, a majority of the tyrosine residues are buried and not exposed to aqueous environment. Guanidine hydrochloride (Gu-HCl) produces partial denaturation with an enhancement of the tyrosine band at 305 nm and shift of the emission maximum from 325 to 330 nm. Analyses of the spectra at different wave lengths of excitation in the presence and absence of the denaturant reveal: (1) three distinct classes of tryptophan residues, as suggested by their emission maxima (2) in the presence of Gu-HCl there are more exposed tryptophan residues in bacteriorhodopsin than in rhodopsin. In contrast to bacteriorhodopsin, the temperature-dependence emission spectra reflects no lipid phase transition; rather, the change is consistent with the thermal denaturation of the rhodopsin molecule.

M-PM-Po74 HIGH RESOLUTION X-RAY DIFFRACTION OF PHOTORECEPTOR MULTILAYERS. Sol M. Gruner, Kenneth J. Rothschild, Noel A. Clark. Department of Physics, Princeton University, Princeton, N.J. 08540;Department of Physics and Physiology, Boston University, Boston, MA, 02215; Department of Physics, University of Colorado at Boulder, Boulder, CO 80309.

Phase seperation in artificially stacked multilayers of isolated bovine retinal rod outer segment membranes has been examined via X-ray diffraction and electron microscopy. Specimens were prepared by isopotential spin-drying followed with partial hydration by equilibration against moist gas streams. Upon dehydration, the lamellar membrane multilayer phase assumes a binary phase composition consisting of concentrated protein containing bilayers interspersed with microdomains of hexagonally packed tubes of lipid in an H_{II} configuration. The H_{II} lattice is geometrically coupled to the lamellar phase with one set of hexagonal crystal planes co-planar to the local membrane lamellae. The hexagonal microdomains bear a striking resemblance to the "paracrystalline inclusions" observed in fast-frozen, intact frog rod outer segments (Corless & Costello, Exp. Eye Res. 32, 217). The lamellae lattice domains are characterized by an unusually small degree of disorder. Sharp lamellar diffraction with a unit cell repeat of roughly 120A (at near total dehydration with 2 bilayers per unit cell) is observed to a resolution of 5A. Comparison of the X-ray diffraction patterns of these specimens with those obtained from highly hydrated disk vesicle multilayers indicates the disk bilayer to be markedly asymmetric. This asymmetry is manifest as a large intensity increase in every other lamellar order when the unit cell repeat shrinks below roughly 150A.

M-PM-Po75 BINDING OF FIBRONECTIN TO PHOSPHOLIPID VESICLES. J.D. Rossi and B.A. Wallace, Department of Biochemistry, Columbia University, New York, N.Y. (Introduced by I.S.Edelman).

The structure of purified human fibronectin in organic, aqueous and detergent solutions has been examined and compared to its structure when bound to phospholipid vesicles. This membrane surface protein binds tightly to phospholipid vesicles of various compositions, as shown by density gradient centrifugation. The binding occurred in the absence of other proteins, or divalent cations and resulted in aggregation of phosphatidyl choline vesicles, which then appeared to fuse upon standing. Circular dichroism spectroscopy indicated the protein adopted different conformations in aqueous-soluble and vesicle-associated forms.
Supported by NIH grant GM 29272 and Jane Coffin Childs Grant #359.

M-PM-Po76 AMILORIDE-INHIBITED SODIUM TRANSPORT INTO RABBIT KIDNEY MEDULLA MICROSOMES BY Edward F. LaBelle and Sun O. Lee (Intr. by Louis Sordahl), The University of Texas Medical Branch, Galveston, Texas 77550.

The medulla of the rabbit kidney has been separated into four subcellular fractions by the procedure of Barnes, L.D., et al. (1974) Endocrinology 96, 119-129. These fractions comprised nuclei, mitochondria, basal-lateral plasma membranes and microsomes. The basal-lateral plasma membrane fraction was shown to be enriched in the marker enzyme Na^+, K^+ATPase, while the microsomal fraction was shown to be enriched in amiloride-inhibited Na^+ transport activity, a potential marker for apical membranes of distal tubular origin. The medulla microsomes were shown to contain only trace amounts of marker enzymes for brush-border vesicles of proximal tubular origin, such as maltase, trehalase, and alkaline phosphatase. Sodium transport into the medulla microsomes was 75% inhibited by amiloride. This Na^+ uptake was shown to represent transport when the uptake process was reversed by the ionophore nigericin and when the process was shown to be sensitive to changes in medium osmolarity caused by alterations in medium sucrose concentration. Sodium transport was complete within 60 min and proportional to the microsomal protein concentration. The effect of amiloride on transport was specific since the similar compound sulfaguanidine failed to affect microsomal Na^+ transport. Amiloride-sensitive Na^+ transport into microsomes was inhibited 70% by decreasing the pH (from 7.0 to 5.9), but was unaffected by the presence of a pH gradient. The kinetics of Na^+ transport could be explained by a simple model, assuming that amiloride lowered the rate of Na^+ entry into the vesicles, but had no effect on the rate of efflux. The failure of amiloride to effect efflux from the vesicles was also demonstrated directly. (Supported by grant from DHHS, NIAMDD, No. AM-25244).

M-PM-Po77 INHIBITIONS OF ANION EXCHANGE INDUCE A TRANSMEMBRANE CONFORMATIONAL CHANGE IN BAND 3. Ian G. Macara & Lewis C. Cantley (Dept. of Biochemistry & Molecular Biology, Harvard University, Cambridge, MA, USA)

Band 3 exchanges anion across the red cell membrane. Exchange is inhibited by large aromatic anions such as the stilbene disulfonates and eosin maleimide, which bind exclusively at the external transport site of the protein. We have prepared sealed inside-out vesicles and right-side-out ghosts from red cells labelled with eosin maleimide, and have used collisional quenching of the eosin fluorescence by Cs^+ to show that the inhibitor becomes exposed to the cytoplasmic side of the cell membrane after reaction at the external transport site. Control experiments established that neither the Cs^+ nor eosin maleimide penetrated the cell membrane. The eosin maleimide had not reacted with sulfhydryl groups on the cytoplasmic domain of Band 3. The partial translocation of the inhibitor across the membrane was found to be accompanied by a conformational change in the protein. The change was detected as an increase in exposure of membrane tryptophan residues to collisional quenchers. A decrease in tryptophan fluorescence intensity, consistent with increased exposure to a polar environment, was also observed upon reaction of membranes with eosin maleimide and upon binding of a non-fluorescent stilbene disulfonate (H_2DBDS). The membrane tryptophan residues accessible to collisional quenchers are all on the cytoplasmic domain, and we suggest that the partial translocation of anion exchange inhibitors across the membrane by Band 3 induces a conformational change in this domain. This work was supported by Grant GM26199 (NIH to L.C.C.). I.G.M. was supported by a grant from the Charles A. King Trust, Boston, MA.

M-PM-Po78 DEVELOPMENT OF A FLUORESCENT ASSAY OF IONOPHORIC ACTIVITY APPLIED TO A NOVEL CHANNEL FORMER FROM E. HYSTOLYTICA. L.M. Loew, SUNY at Binghamtom, Binghamton, NY 13901; C. Gitler, I. Rosenberg, The Weizmann Institute of Science, 76100 Rehovot, Israel; and E.C. Lynch, Albert Einstein College of Medicine, Bronx, NY.

Entamoeba Hystolytica contains remarkable channel forming activity ("amoebaphore") which spontaneously incorporates into planar lipid bilayers. Under voltage clamp of a bilayer, the amoebaphore has a small selectivity for K^+ but also allows Na^+ and, to a much lesser extent, Cl^- to flow through the membrane. A partial purification of the amoebaphore was made possible by the development of a simple assay which should be of general applicability for the detection of non-selective ionophores. It involves following, via the fluorescent probe di-S-C_2(5), the rate of collapse of a valinomycin mediated K^+-diffusion potential across asolectin liposomes which have been exposed to the amoeba fractions. The activity can be expressed in units of equivalent concentrations of a well characterized ionophore such as gramicidin. The amoebaphore is a protein associated with a discrete particulate fraction from whole amoeba lysate. (Supported by USPHS Grant GM25190, The Rockefeller Foundation, and a NIH Research Career Development Award, CA677, to L.M.L.)

M-PM-Po79 QUENCHING OF APOLIPOPROTEIN A-I AND ALKYLINDOLE FLUORESCENCE BY OXYGEN IN SOLUTION AND IN LIPID-PROTEIN COMPLEXES. W.W. Mantulin and H.J. Pownall, Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030 and D.M. Jameson, Department of Biochemistry, University of Illinois, Urbana, Illinois 61801.

Apolipoprotein A-I (apoA-I) is the major protein component of human plasma high density lipoprotein. The isolated, lipid-free protein is soluble, but has a tendency to self-associate in solution. ApoA-I will spontaneously form lipoprotein recombinants with phospholipid. We have examined the temperature dependent effects of self-association and complex formation with dimyristoylphosphatidylcholine (DMPC) on the efficiency of apoA-I (tryptophan) fluorescence quenching by oxygen. Dynamic, diffusion controlled, quenching of fluorescence is described by the Stern-Volmer equation, with k* the bimolecular quenching constant. The quenching of apoA-I fluorescence is independent of self-association, and only slightly dependent on temperature. In association with DMPC, k* increases and shows the temperature dependence (33°>20°>26°). Quenching of an alkylindole in DMPC does not exhibit a marked temperature dependence. From this data, we conclude that 1) self-association of apoA-I does not impede the diffusion of oxygen to tryptophan residues buried in the protein core, 2) association of apoA-I with DMPC raises the apparent k* by increasing the localized concentration of oxygen and 3) the temperature dependence of k* in apoA-I/DMPC complexes relfects protein conformational change. [Supported by a grant from the American Heart Association, Texas Affiliate,Inc., and grants from the National Institutes of Health (HL17269 and HL26250)]

M-PM-Po80 PROPERTIES OF TWO PROTEOLYTIC FRAGMENTS OF COLICIN E1. ‡J.R. Dankert, K.R. Brunden, W.A. Cramer, M. Hermodson†, F.S. Cohen‡, and J.O. Bullock‡, Depts of Biological Sciences and Biochemistry†, Purdue University, W. Lafayette, IN 47907, and Dept. of Physiology‡, Rush Medical College, Chicago, IL 60612.

Digestion of colicin E1 with dilute trypsin results in the generation of a relatively hydrophobic M_r = 20,000 trypsin-resistant peptide fragment. Alignment of the N-terminal sequence with the complete amino acid sequence of the colicin E1 molecule obtained by Chan and Lebowitz (manuscript in preparation) shows that the fragment occupies all, or almost all, of the C-terminal section of the molecule. This fragment, incorporated into DMPC vesicles in a cholate dispersion, behaves similarly to colicin E1 in being able to dissipate a K^+-diffusion potential above or below the Tm of DMPC vesicles. The fragment did not show measurable binding to colicin receptor sites on the cell surface, but was much more efficient than colicin E1 in its ability to inhibit proline transport by inner membrane vesicles of E. coli. Using planar membranes formed from monolayers of asolectin, the voltage-dependent macroscopic conductance properties of the M_r = 20,000 fragment are qualitatively the same as those of colicin E1. Voltage-dependent insertion of E1 and this fragment into sonicated asolectin vesicles has also been observed. The data imply that a membrane channel-forming function of the molecule is located in this 20,000 molecular weight region at the C-terminal end of the colicin molecule. A second M_r = 40,000 fragment, which may include the receptor-binding domain of the colicin, has been isolated after treatment with thermolysin. It competes for E1 receptor sites, does not kill cells, inhibits proline transport of vesicles, and its macroscopic conductance properties do not mimic those of E1. (Supported by NIH grants GM18457 and GM27367.)

M-PM-Po81 THE EFFECT OF CHOLESTEROL ON THE BINDING OF TRANSFERRIN TO ITS RECEPTOR. A RECONSTITUTION STUDY. **Marco-Tulio Nunez and Jonathan Glass. Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory of Beth Israel Hospital, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA.**

Purified transferrin receptors isolated form rabbit reticulocytes were incorporated into egg phosphatidylcholine vesicles containing varying amounts of cholesterol. The reconstituted vesicles had three distinct properties:

1.- The binding of transferrin to the reconstituted vesicles exhibited the two components characteristic of transferrin binding to erythroid cells, a specific component that saturates at $1\text{-}2\times10^{-6}$M transferrin, and a non-saturable, non-specific component.

2.- Positive cooperativity of transferrin binding was observed at low molar ratios of cholesterol to phosphatidylcholine (C/P). However, the cooperativity diminished on increasing C/P ratios, and was no longer seen at a C/P ratio equal to that found in circulating red blood cells.

3.- The amount of specific transferrin binding to the reconstituted vesicles was inversely proportional to the C/P ratio. Binding of transferrin at a C/P molar ratio of 1.0 was only one third of the binding observed in vesicles containing no cholesterol.

These results indicate that membrane cholesterol levels may play a significant role in the expression of transferrin receptors in erythroid cells. **Supported by USPHS grant AM17148 and RCDA AM00513 (J. G.)**

M-PM-Po82 INTERACTION OF LECTINS WITH SPIN LABELED GLYCOPROTEINS
Bill C.P. Kwok and Frank R. Landsberger, The Rockefeller University, New York, New York. 10021

To investigate the interaction of lectins and enveloped viruses with the carbohydrate of glycoproteins, spin labeled fetuin was prepared as a model system. The neuraminic acid (nana) of fetuin was subjected to periodate oxidation and tritiated borohydride reduction in the presence of tempamine (TA) to give TA-nana-fet. By acid hydrolysis, neuraminidase treatment, and salt precipitation, this procedure produces a nana specific probe which can be used to investigate the environment of nana. When TA-nana-fet was treated with wheat germ agglutinin (WGA), immobilization of the spin signal was observed and is reversible by the addition of native fetuin. Spin labeled asialo fetuin (TA-gal-fet) prepared by neuraminidase and galactose oxidase treatment failed to precipitate WGA and signal immobilization was not observed. Ricin (RCA) did not seem to recognize TA-gal-fet despite the terminal galactoses suggesting that the 6-OH position is important for RCA binding. Thus, each substrate must be carefully tested with respect to the relevant lectin to assure that spin labeling does not affect the binding specificities of the oligosaccharide.

M-PM-Po83 PHASE AND MODULATION FLUORESCENCE STUDY OF LIPID STRUCTURE AND DYNAMICS IN Ca^{2+}-ATPASE SARCOPLASMIC RETICULUM VESICLES. David A. Barrow, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27514.

The phase-resolved fluorescence of 1,6-diphenyl-1,3,5-hexatriene (DPH) has been used to investigate the effects of the Ca^{2+}-ATPase on the organization and motion of the lipid molecules in sarcoplasmic reticulum vesicles. Previous steady-state fluorescence measurements on this system have suggested a model involving a disrupted secondary layer of lipid outside the so-called "boundary" or "annulus" layer immediately adjacent to the protein (Lentz, B.R., B.M. Moore, C. Kirkman, G. Meissner, 1981, Biophysical Discussions, in press). The predictions of this and other models of protein-lipid interaction in the sarcoplasmic reticulum are evaluated in light of new evidence obtained by the phase and modulation techniques.

In addition, methods are presented which facilitate the use of commercially available phase and modulation lifetime fluorometers for resolving fluorescence lifetime heterogeneity. These methods include data acquisition improvements and the use of isochronic reference standards for the reduction of instrumental errors in the measurement of fluorescent lifetimes on the order of ten nanoseconds. (Supported by NSF PPM 79-22733 to Barry R. Lentz who is an Established Investigator of the American Heart Association.).

M-PM-Po84 ENCAPSULATION OF HEMOGLOBIN IN PHOSPHOLIPID VESICLES Bruce P. Gaber, Paul Yager, James P. Sheridan and Eddie L. Chang; Biomolecular Optics Section, Optical Probes Branch, Code 6510, Naval Research Laboratory, Washington, D.C. 20375, USA

As a first step in developing a blood surrogate, we have succeeded in encapsulating hemoglobin in large unilamellar vesicles of egg phosphatidylcholine. Purified hemoglobin solutions ($\sim$ 100mg/ml) were used to disperse the phospholipid from a lyophilized powder. The dispersion was then extruded through a series of polycarbonate membranes of defined pore size and passed through a Sepharose CL 6B column to separate the vesicles from unencapsulated protein. The vesicles were found to be homogeneous and in the range 2500-3000Å in diameter as determined by photon correlation spectroscopy. Monitoring the absorption spectrum in first derivative mode allowed observation of reversible oxygenation of the encapsulated hemoglobin. The vesicular preparations are stable for weeks at 4°C. (Supported in part by the Office of Naval Research.)

M-PM-Po85 MEASUREMENT OF THE RATE OF AROMATIC RING MOTIONS IN PROTEINS THROUGH ^2H QUADRUPOLE ECHO NMR LINESHAPES. David M. Rice, Paul Gingrich, Judith Herzfeld, and Robert G. Griffin, Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge MA 02139 and Biophysical Laboratory, Harvard Medical School, Boston MA 02115.

The ^2H quadrupole echo NMR lineshape is sensitive to C-D bond motions, and can be used to measure the rate of amino acid motions in proteins and peptides. Crystalline d_5-phenylalanine·HCL was found to undergo a rotational jump of 180° between the two symmetric ring positions at rates of $10^3 s^{-1}$ to $10^6 s^{-1}$ between 30°C and 165°C, and this system has served as a good model for the analysis of the resulting ^2H lineshapes. Bacteriorhodopsin of the Purple Membrane was labelled with d_5-phenylalanine through enrichment of the Halobacterium growth medium. Through comparison of the ^2H results with those of crystalline phenylalanine, it was found that the motional behavior of most phenylalanines of the Purple Membrane is alike, and that the jump rate for the rings of Purple Membrane is between $10^3 s^{-1}$ and $10^6 s^{-1}$ between -30°C and +30°C. The activation energy for crystalline phenylalanine (E_a = 17 kcal) is similar to that of the more rigid rings of globular proteins while in Purple Membrane E_a is noticeably smaller.

(Supported by NIH Grants GM23316, GM23289, RR0995, and a Faculty Research Award to J.H. from the American Cancer Society.)

M-PM-Po86 FLUORESCENCE ENERGY TRANSFER AND STEADY-STATE ANISOTROPY OF DIPHENYLHEXATRIENE IN VESICLES CONTAINING BACTERIORHODOPSIN. M. Rehorek, N.A. Dencher and M.P. Heyn, Dept. of Biophys.Chem., Biozentrum, Basel, Switzerland.

Fluorescence energy transfer between the donor diphenylhexatriene (DPH) and the acceptor retinal and fluorescence depolarization of DPH are used to obtain information on the location of the retinal chromophore inside bacteriorhodopsin and on the effect of this intrinsic membrane protein on the order and dynamics of the lipid phase. Increasing the surface concentration of acceptors by raising the protein to lipid ratio, leads to a decrease in the mean fluorescence lifetime by up to a factor of four. When the acceptor concentration is reduced at fixed protein to lipid ratio by photochemical destruction of retinal, the lifetime increases and reaches approximately the value observed in protein-free vesicles when the bleaching is complete. The shape of the decay curve and the dependency of the mean lifetime on the surface concentration of acceptors are in agreement with theoretical predictions for a two-dimensional random distribution of donors and acceptors. From this analysis a distance of closest approach between donors and acceptors of about 23 Å is obtained, suggesting that retinal is deeply buried in the interior of bacteriorhodopsin. In the absence of energy transfer (bleached vesicles), the steady-state fluorescence anisotropy $\bar{r}$ of DPH is considerably lower than in the corresponding unbleached vesicles, indicating that the effect of energy transfer must be taken into account when interpreting $\bar{r}$ in terms of order and dynamics.

M-PM-Po87 PLANAR AGGREGATION OF BACTERIORHODOPSIN IN A FLUID BILAYER OCCURS ONLY AT EXTREMES OF THICKNESS. B.A. Lewis and D.M. Engelman, Yale University, New Haven, CT 06511.

How can a single "fluid" membrane develop the regions of specialization which are observed in many cell membranes? We have examined the influence of hydrophobic interactions between transmembrane proteins and lipids on protein planar distribution, asking the following questions: If the transmembrane dimension of a protein hydrophobic surface differs from the equilibrium thickness of its bulk lipid environment, will protein monomers aggregate to decrease the protein-lipid contact surface area? If so, how large must the difference be to induce aggregation?

Freeze-fracture electron microscopy was used to observe the planar organization of the intramembranous particles (IMPs) in vesicles made from delipidated bacteriorhodopsin (BR) and synthetic lecithins; lipid bilayer thickness was determined by X-ray diffraction. Using lecithins with acyl chains from di-10:0 to di-24:1, the thickness of the bilayer hydrocarbon region above Tm was varied from 16 to 38 Å. The hydrophobic surface of BR has a transmembrane dimension of about 30 Å.

Only the thin- (10:0) and thick- (24:1) bilayer samples showed any IMP aggregation above Tm. These results are consistent with a high degree of elasticity of the bilayer, especially for stretching; compression may be more costly energetically. Thus if a protein were designed to self-associate in a fluid membrane by having a hydrophobic surface geometry which differs from that of its lipid environment, it would need a surface at least 90% wider or at least 20% narrower than that of the unperturbed lipid bilayer to produce appreciable aggregation. A further result is that the IMPs in the thickest-bilayer sample (24:1) are probably trimers of BR, even above Tm.

We thank Martin Caffrey and Gerald Feigenson for generous gifts of di-22:1 and di-24:1 lecithin.

M-PM-Po88 CONFORMATION OF MEMBRANE PROTEINS DETERMINED FROM THE SPATIAL DISTRIBUTION OF TRYPTOPHAN. Kleinfeld, A.M., Lukacovic, M., Matayoshi, E.D. and Holloway, P. Biophysical Laboratory, Harvard Medical School, Boston, MA 02115 and Max Planck Institut Fur Biophysical Chemie, Gottingen, W. Germany and Dept of Biochemistry, University of Virginia, Charlotsville,VA.

We have measured energy transfered from tryptophan (TRP) of the band 3 protein of human erythrocytes and b5 from rabbit, to a series of n-anthroyloxy (AO) fatty acid probes. We used a preparation of ghosts enriched in band 3 and b5 reconstituted into small sonicated lipid vesicles. Since the bilayer depth of the AO are known it is possible to map out the spatial distribution of Trp. The b5 was used to test this approach since it has a single fluorescent TRP. In b5 vesicles the energy transfer of n-AO increases from n=2 to about n=9 and is then constant to n=16. These results can be modeled by a single TRP at 20 Å from the bilayer surface in excellent agreement with the work of Flemming et al 1979, Biochemistry, 18, 5458. For band 3 vesicles the energy transfer decreases from n=2 to n=6,7 and then rises continuously to n=16. To model these observations a bimodal Trp distribution is necessary, in which several TRP extend about 10 Å into the cytoplasm and a larger group are located near the outer surface of the membrane. To determine the effect of the dipole orientation factor, differential polarized lifetimes of the AO probes were measured. We find that the AO moiety can be considered to be rotating rapidly (10^8 radians/sec) and nearly isotropically (cone half angle > 75°). A theoretical description has been developed for the orientation factor corresponding to a donor dipole confined to a cone and an isotropic acceptor. Calculations of the energy transfer efficiency for the geometry appropriate to a membrane protein surrounded by the AO acceptors, demonstrate that the uncertainty associated with the donor dipole will not, significantly change the distribution. Work supported by grant GM26350 NIH and JRFA-15 Am. Can. Soc.

M-PM-Po89 CHARGE CLUSTERS AND THE ORIENTATION OF MEMBRANE PROTEINS.
John N. Weinstein, Robert Blumenthal, Christoph Kempf, Jos Van Renswoude, and Richard D. Klausner. Laboratory of Mathematical Biology, NCI, NIH, Bethesda, MD 20205.

Recent studies in our laboratory on a hepatic receptor protein and on melittin indicate that electrostatic forces can influence the position and orientation of a protein inserted into a membrane. These observations led us to predict that transmembrane proteins would have clusters of positive and negative residues at the cytoplasmic and extra-cytoplasmic ends, respectively, of their hydrophobic transmembrane segments. This asymmetry would be preferred on the basis of the inside-negative potentials across plasma membranes. The prediction is borne out by the few transmembrane proteins (glycophorin, M13 coat protein, H-2K^b, HLA-A2, HLA-B7, and mouse Igμ heavy chain) for which we have sufficient information on both sequence and orientation. In addition to potentials measurable with electrodes, the "microscopic" membrane potential reflects surface charge effects, which we analyze using a generalized form of Stern equation. Dipole potentials within the membrane would increase the potential energy barrier for passage of positive residues across the membrane out of the cytoplasmic compartment, again consistent with the observed protein orientations. These considerations lead us to two hypothetical models of protein translocation, the first involving initial interaction of the transmembrane segment with the membrane; the second assuming initial interaction of a leader sequence.

M-PM-Po90 THE EFFECT OF CYTOCHROME b_5 ON PHOSPHOLIPID TRANSBILAYER DISTRIBUTION IN MODEL MEMBRANES,
J.R. Nordlund, C.F. Schmidt, P.W. Holloway and T.E. Thompson, Dept. of Biochemistry, University of Virginia, Charlottesville, 22908.

We have attempted to test a model for the generation and maintenance of phospholipid transbilayer asymmetry in biological membranes in which the insertion of a protein into pre-formed bilayers, (a) creates a new transbilayer distribution via interactions with the phospholipids, and (b) facilitates transbilayer movement of the lipids. The protein chosen was cytochrome b_5, which is soluble without detergents in aqueous solution, but inserts spontaneously into vesicles. The vesicles were composed of 30 mole percent phosphatidylethanolamine (PE) in phosphatidylcholine (PC). Both small, unilamellar, asymmetric PE vesicles, prepared by sonication, and large, unilamellar, symmetric PE vesicles, prepared by ethanol injection, were used. The vesicles were size fractionated by centrifugation, incubated with cytochrome b_5, and the PE distribution determined by trinitrobenzenesulfonate labeling. Mole ratios of lipid to protein as low as 280 for the small vesicles and 100 for the large vesicles could be studied without breakdown of the permeability barrier. At every phospholipid to protein ratio examined, the PE distribution was not significantly different from that in control vesicles without protein. Identical results were obtained for vesicles in which the hydrophilic portion of cytochrome b_5 was removed by trypsin treatment before labeling. Ethanol (20% v/v) was added to some preparations in an attempt to facilitate rearrangement, but no changes were observed. These results indicate either that the phospholipid distribution is not perturbed by the addition of cytochrome b_5, or that the distribution is kinetically trapped.
(Supported by U.S.P.H.S. Grants GM-14628, GM-23573, GM-17452, and GM-23858.)

M-PM-Po91 BINDING OF APOCYTOCHROME C TO LIPID VESICLES
Mark E. Dumont and Frederic M. Richards, Department of Molecular Biophysics & Biochemistry. Yale University, New Haven, Connecticut 06511

Apocytochrome c (cytochrome c without the heme) is synthesized on free cytoplasmic ribosomes without any cleaved "leader" sequence and transported post-translationally to its functional site between the inner and outer mitochondrial membranes. We are investigating the interactions between apocytochrome c and liposomes as a model for such transport. Large unilamellar vesicles of the approximate lipid composition of the outer mitochondrial membrane bind apocytochrome c, but not holocytochrome c, upon incubation at 37°C for two hours (pH7.2). This binding occurs even in 0.5M NaCl. To measure the binding, free protein was separated from vesicles by agarose gel chromatography and by centrifugation on sucrose density gradients. Upon trypsin digestion of apocytochrome c, a large portion of protein bound to vesicles was protected against proteolysis relative to protein in solution. The hydrophobic-phase photoactivated label, [^{125}I] 3-trifluoromethyl-3 (m-iodophenyl) diazirine (TID) (1), labels apocytochrome c, but not holocytochrome c, that has been incubated with liposomes. Thus, we have obtained evidence that apocytochrome c interacts strongly with lipid bilayers in the absence of any protein receptors for transport. Additional experiments using photo-reagents and proteolytic enzymes are being carried out to determine whether bound apocytochrome c actually spans the liposome membranes. The specificity of the interaction for apocytochrome c under the condition used here suggests the need for caution in interpreting differences between the behavior of precursor and mature proteins as evidence for specific protein-receptor interactions. (1) Brunner, J. and Semenza, G., Biochemistry, in press.

M-PM-Po92 EFFECT OF PRESSURE AND TEMPERATURE ON $(Na^+ + K^+)$-ATPase. P.L.-G. Chong, D. M. Jameson, P. A. G. Fortes and G. Weber (Department of Biochemistry, University of Illinois, Urbana, IL. and Department of Biology, University of California, San Diego, LaJolla, CA)

The inhibition of $(Na^+ + K^+)$-ATPase activities by pressure has been reported by Smedt et al. ((1979) Biochem. Biophys. Acta, 556, 479) who suggested that it was due to changes in membrane fluidity. Dog kidney $(Na^+ + K^+)$-ATPase was purified by the method of Jørgensen. The enzyme activities were measured under hydrostatic pressures in the range of 10^{-3} to 2.5 Kbar. The K-pNPPase activity decreases with pressure with an apparent activation volume of 17 ml/mole at 35.5° C. The dT/dP value for this enzyme reaction is about 22° $Kbar^{-1}$. The Na-K ATPase activity also decreases with pressure with a dT/dP value estimated to be 16 to 20° $Kbar^{-1}$. The observed dT/dP values suggest that both activities are linked to lipid-involving processes. The enzyme activities were then correlated with membrane fluidity by measurements of the change of stationary fluorescence polarization of diphenylhexatriene (DPH) in ATPase membranes under pressure which itself showed dT/dP = 18 to 21° $Kbar^{-1}$. The pressure effects on enzyme activities and DPH polarization are reversible. In the range of 10^{-3} to 1 Kbar and 23 to 35.5°C, to any two pairs of values of temperature and pressures, (T_1, P_1 and T_2, P_2), giving similar enzyme activity, there correspond two very close DPH polarization values indicating a direct correlation of enzyme activities with membrane fluidity. Energy transfer between anthroylouabain and NBD-amino-pyranosildigitoxigenin and polarization of their fluorescence did not change appreciably with pressure in the range of 10^{-3} to 2.5 Kbar. (This work is supported by NIH RR-08135 and HEW PHS GM 11223.)

M-PM-Po93 THE EFFECTS OF THE PERTURBATION OF THE COUNTERION DISTRIBUTION ON THE MOVEMENT OF MEMBRANE CONSTITUENTS IN AN ELECTRIC FIELD. James R. Rabinowitz (Intr. by C. Blackman), U.S. Environmental Protection Agency, HERL, EBD (MD-74), Research Triangle Park, NC 27711.

Various investigators have demonstrated that externally applied electric fields perturb the distribution of charged components in biological membranes. In this study the effect of the external field on the distribution of ions, too close to the cell surface to be part of the closed circuit current, is considered and the electric field at the cell surface due to the polarization of this ion distribution is calculated. In the model used for these calculations, the cell is assumed to be an immobile sphere or hemisphere with charged molecules in or on the surface. Some of these charged molecules are free to move within the cell surface. Gouy-Chapman theory is used for the unperturbed ion distribution. It has been found that the initial increment of the field parallel to the cell surface resulting from this polarization is $-AE_o\sin\theta_o$ at any point (a,θ_o,ϕ_o) on the cell surface, where E_o is the magnitude of the external electric field applied in the $\hat{z}$ direction, $qaE_o \ll kT$, a is the radius of the cell. For 0.15 molar monovalent salt, A varies from 0.16 to 1.20 as the surface potential of the cell varies between 10 and 60 mV. The calculations have been performed with a screened potential, and A may be greater than indicated. For larger fields, $qaE_o \gtrsim kT$, the angular dependence is more complicated. These calculations provide an explanation for the accumulation of negatively charged intramembraneous particles at the side of the cell near the negative pole and indicate this effect must be considered in studies of the in situ electrophoresis of membrane constituents. Other similar calculations and the use of discrete charges will be discussed.

M-PM-Po94 STRUCTURAL STUDIES OF A FUNCTIONAL CYTOCHROME c-PHOTOSYNTHETIC REACTION CENTER MEMBRANE. J.M. Pachence, P.L. Dutton, and J.K. Blasie, University of Pennsylvania, Phila., PA 19104 and Columbia University, N.Y., NY 10032.

Structural information on cytochrome c binding to photosynthetic reaction center/phospholipid membranes has been derived using neutron diffraction. A reconstituted membrane system was formed from either deuterated or protonated reaction center (RC) protein (isolated from the photosynthetic bacteria Rps. sphaeroides R26 mutant) and pure egg phosphatidylcholine (EPC) membrane bilayers. Partially dehydrated, oriented membrane multilayers were formed from a dispersion of EPC/RC membrane vesicles and horse heart cytochrome c (with a molar ratio of EPC/RC=100 and Cytochrome c/RC=0.8 in the multilayers). Neutron scattering membrane profiles were determined from the lamellar neutron diffraction of oriented membrane multilayers containing either 1) cytochrome c/EPC/deuterated RC or 2) cytochrome c/EPC/protonated RC. The difference between these two scaled profile structures directly determined the reaction center profile within the cytochrome c/EPC/RC membrane. This protein profile was compared to the reaction center profile within a membrane without cytochrome c (previously derived); differences between the two RC profiles were within the significance of the experiment. A cytochrome c/EPC profile was also derived, showing that the distribution of lipid on opposite sides of the membrane was shifted with the addition of cytochrome c. Finally, model profiles which were calculated using the neutron diffraction information were compared with X-ray diffraction profiles of the cytochrome c/EPC/RC membranes to determine the position of cytochrome in the membrane profile to 10 Å resolution. Supported by NIH grant GM 27309.

M-PM-Po95THE LOCATION OF REDOX CENTERS IN THE PROFILE STRUCTURE OF RECONSTITUTED MEMBRANES CONTAINING A FUNCTIONAL PHOTOSYNTHETIC REACTION-CENTER: CYTOCHROME c COMPLEX BY RESONANCE X-RAY DIFFRACTION. J. K. Blasie, J. Pachence, P. L. Dutton, J. Stamatoff, P. Eisenberger, and G. Brown, University of Pennsylvania, Philadelphia, PA 19104, Bell Telephone Laboratories, Murray Hill, NJ 07974 and the Stanford Synchrotron Radiation Laboratory, Stanford, CA 94305

We have utilized the effects of resonance X-ray scattering from iron and cobalt atoms on the lamellar X-ray diffraction from oriented multilayers of reconstituted membranes containing a functional photosynthetic reaction-center: cytrochrome c complex to determine the location of (a) the iron atom of the iron-quinone electron acceptor of the reaction-center in the membrane profile to an accuracy of ± 1 Å and (b) the cobalt atom of the cobalt-heme of cytochrome c in the membrane profile to ± 1 Å. The separate profile structures of the reaction-center, cytochrome c and phospholipid molecules have been determined independently within these same reconstituted membranes using X-ray and neutron diffraction (1). The resonance diffraction experiment utilized a focused beam-line at the Stanford Synchrotron Radiation Laboratory, a Huber 4-circle diffractometer and θ-2θ scans to collect the lamellar diffraction data as a function of incident X-ray energy about the iron and cobalt K-absorption edges. A model refinement analysis of these data provided the metal atom locations in the membrane profile.

(1) Pachence, et al., BBA, 549, 348-373 (1979) and 635, 267-283 (1981).

M-PM-Po96 SPIN-LABELED NADH (SL-NADH) FOR MOTIONAL STUDIES OF D-β-HYDROXYBUTYRATE DEHYDROGENASE (BDH). *Thomas Fritzsche, J. Oliver McIntyre, *Wolfgang Trommer and Sidney Fleischer. Vanderbilt University, Nashville, TN and *University of Stuttgart, W. Germany.

BDH is a lecithin-requiring enzyme purified from beef heart mitochondria. The lecithin is essential for coenzyme (NADH) binding. We have measured NADH binding to the active BDH-mitochondrial phospholipid (MPL) complex by fluorescence energy transfer and have now shown that: (1) the NADH dissociation constant (K_D) for binding to BDH decreases sharply with increasing pH from ~40μM at pH 6.2 to ~7μM at pH 8.5 with a pK at pH 6.8 (Dixon plot); (2) the K_D at pH 8.0 decreases sharply with increasing salt (LiCl or LiBr) concentration to ~5μM at 50mM salt but higher salt concentrations up to 200mM do not further alter K_D significantly. Enhanced NADH binding corresponds with a marked decrease in BDH activity suggesting that the rate of β-hydroxybutyrate oxidation is influenced by the off rate of NADH. (3) 40mM monomethylmalonic ester (MMM), a product analogue, enhances NADH binding (K_D ~1μM); (4) SL-NADH, spin-labeled on the N6 of the adenine moiety, binds to BDH with K_D (~1.5μM) similar to that for NADH. EPR and ST-EPR spectra show that the spin-label of SL-NADH is motionally constrained when bound to BDH. Deuterated SL-NAD(H) binds to BDH and gives enhanced EPR spectral resolution. Similar EPR spectra are obtained for either SL-NADH or SL-NAD^+ bound to BDH-MPL both in the presence and absence of MMM and for SL-NAD^+ with SO_3^{2-}. EPR and ST-EPR spectral parameters, although similar, indicate distinct differences in motional characteristics compared with SL-maleimide covalently bound to BDH. Both SL-NAD(H) and SL-maleimide afford unique site labeling of BDH and hence intramolecular distances can be estimated.

[Supported by NIH AM 21987 and by Deutsche Forschungsmeinschaft]

M-PM-Po97 FLUORESCENCE LABELING OF THE Na,K ATPase BETA SUBUNIT. Jonathan A. Lee and P.A. George Fortes, Department of Biology, University of California, San Diego, La Jolla, California 92093.

Na,K ATPase is composed of a catalytic α subunit and a glycoprotein β subunit of unknown function. To study the β subunit by spectroscopic techniques, we synthesized new fluorescent probes and developed a method to couple them specifically to the oligosaccharide portion of the β subunit of purified dog kidney Na,K ATPase. The labeling procedure involved enzymatic oxidation of the oligosaccharide portion of the β subunit, reaction of the resulting aldehydes with fluorescent hydrazides, and reduction with $NaBH_4$. The commercially available fluorescent hydrazides and aryl amines surveyed did not label the β subunit. β subunit labeling required fluorescent reagents with a four to five atom spacer between the fluorophore and hydrazide moieties. Fluorescein and 1,5-dansyl hydrazides were synthesized with an appropriate spacer group. After reaction with Na,K ATPase a single fluorescent band, corresponding to the β subunit, was detected on SDS-PAGE. Both compounds specifically labeled the β subunit without loss of enzymatic activity or cardiac glycoside sites. Significant energy transfer was detected between the dansylated oligosaccharide portion of the β subunit and NBD-aminopyranosyl digitoxigenin specifically bound to the cardiac glycoside site on the α subunit. This labeling procedure, in conjunction with fluorescence energy transfer, may be useful to study α-β interactions, structure and dynamics of the β subunit and relative conformational changes between the α and β subunits. (Supported by NIH grants HE-20262 and RR-08135).

M-PM-Po98 THE ACTIVE SITE STRUCTURE OF (Na,K)-ATPase: LOCATION OF A SPECIFIC FLUORESCEIN ISOTHIOCYANATE REACTIVE SITE. C.T. Carilli*, R.A. Farley+, D. Perlman*, and L.C. Cantley*. *Harvard University, Cambridge, MA 02138 and +U. of Southern California, Los Angelos, CA 90033.

At low concentrations fluorescein-5'-isothiocyanate (FITC) specifically inactivates Na^+- and K^+-dependent adenosine triphosphatase {(Na,K)-ATPase} (Karlish et al., 1979, Nature 282, 333-335). The site of modification of purified dog kidney (Na,K)-ATPase by FITC has been investigated by enzymatic cleavage and fluorescence resonance energy transfer. FITC reacts with a stoichiometry of approximately one site per ATP binding site, and causes ATP-protectable inactivation of ATP hydrolysis without affecting hydrolysis of a smaller substrate, p-nitrophenyl phosphate. The modified site is on the carboxy-terminal 58,000 dalton tryptic fragment of the large subunit of the ATPase, near the site where chymotrypsin cleaves in the presence of ouabain. The site of phosphorylation by ATP, however, is on the amino-terminal 40,000 dalton tryptic fragment. Using ^{3}H-anthroyl ouabain (P.A.G. Fortes, 1977, Biochem. 16, 531) as energy donor and covalently bound FITC as acceptor, the distance between the ouabain and FITC sites was measured by fluorescence resonance energy transfer. The distance obtained was ∿74 Å (66-83 Å). Since ouabain binding affects the conformation at the FITC binding site, this result demonstrates propagation of conformational changes through the protein for at least 66 Å.

M-PM-Po99 LOCAL ANESTHETIC INHIBITION OF F_1-ATPase AND OTHER ENZYMES. G. Vanderkooi, D. Chignell, B. Chazotte, and H. Mohd-Sidek, Chemistry Department, Northern Illinois University, DeKalb, Illinois 60115.

We have measured the inhibitory potencies of local anesthetics (e.g., procaine, lidocaine, tetracaine, dibucaine, etc.) on the activity of several membrane and water soluble enzymes. All tested segments of the mitochondrial electron transport chain were inhibited (BBA 636 (1981) 153). Mitochondrial F_1-ATPase is also inhibited, both in the membrane-bound and lipid-free states, by similar concentrations of anesthetics as give nerve block (BBA 635 (1981) 200). A linear relationship was found between the logs of the octanol/water partition coefficients and the concentrations required for 50% inhibition of F_1. Ultracentrifugation and electrophoresis showed that 1.0 mM tetracaine partially dissociated F_1, but the enzyme inhibition by tetracaine was completely reversible. Rat brain synaptosomal acetylcholinesterase is inhibited by local anesthetics in the same concentration range as F_1. Several soluble enzymes were uninhibited or weakly inhibited by local anesthetics, including ribonuclease, lactic dehydrogenase, pyruvate kinase, and glutamate-oxaloacetate transaminase. We conclude that the tertiary amine anesthetics are a heretofore unrecognized class of reversible protein structure perturbants; these may be useful in general studies of protein stability. This work supports the hypothesis that direct protein effects are the basis of anesthetic action. (Supported in part by a grant from the American Heart Association.)

M-PM-Po100 BOUNDARY LIPID CHARACTERIZATION IN CYTOCHROME b_5-DMPC. E. Freire, T. Markello and P.W. Holloway, Departments of Biochemistry, Univ. of Tennessee, Knoxville, TN 37916 and Univ. of Virginia, Charlottesville, VA 22908.

We have studied the interactions of cytochrome b_5 and dimyristoyl phosphatidylcholine (DMPC) using high sensitivity scanning calorimetry and fluorescence spectroscopy. Addition of cytochrome b_5 to large (700 Å) single lamellar vesicles causes a downward shift in the lipid phase transition temperature, a broadening of the excess heat capacity function and a decrease in the enthalpy change, ΔH, associated with the lipid phase transition. The dependence of this decrease in ΔH on the protein/lipid molar ratio indicates that cytochrome b_5 prevents ~ 27 lipid molecules from undergoing the lipid phase transition. This number is similar to the number of lipid molecules required to cover the perimeter of one protein molecule. Fluorescence energy transfer between cytochrome b_5 and pyrene decanoic acid indicates that, in the liquid crystalline phase, the protein and the lipid probes are uniformly distributed within the bilayer plane. In the gel phase pyrene decanoic partitions into the boundary layer lipid causing a dramatic decrease in the fluorescence intensity of cytochrome b_5. The magnitude of this change is a function of the composition and the concentration of lipid probes. This peculiar partitioning of the lipid probes suggest that the boundary lipids are in a disordered state. Analysis of the fluorescence energy transfer experiments using a newly developed method of Monte Carlo calculations also indicate that the boundary layer of lipid is only one lipid thick. (Supported by NIH Grant GM-27244).

M-PM-Po101 SMALL HYDROPHOBIC BIFUNCTIONAL REAGENTS ALTER MEMBRANE-ASSOCIATED EVENTS OF INSULIN ACTION. H.J. Goren, J. Gohill, R. Hughes, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Difluorodinitrobenzene (I), di-isothiocyanate (II), 4-fluoro-3-nitrophenyl azide (III) and p-azidophenacyl bromide (IV) are small hydrophobic bifunctional reagents which we have used to study membrane-associated events of insulin action. Isolated rat adipocytes were exposed to the 4 bifunctional reagents for 45 s, 37°C; conditions which excluded intracellular reactions. At 100 μM, I completely inactivated the cell's metabolic activity (glucose oxidation - basal and insulin stimulated), IV inactivated the cell's activity 75%, III inactivated the cell's activity 50%, while II increased the cell's activity 50%. Inactivation of the cell's glucose oxidation activity was not due to reaction with the insulin receptor since total ^{125}I-insulin bound was unchanged in I-treated adipocytes and insulin prebound to the fat cells did not protect against I inactivation; I is representative of the inactivating-type of hydrophobic cross-linking reagents. Neither did these reagents react with the glucose carrier since neither cytochalasin B nor glucose protected against inactivation of fat cell metabolism by I. II increased adipocyte basal and insulin-stimulated glucose oxidation in a dose-dependent manner. II-treated adipocytes (100 μM) demonstrated a Scatchard plot for insulin binding parallel and to the right of Scatchard plots for controls. These results suggest that hydrophobic bifunctional reagents whose reactive sites are close together can alter fat cell glucose metabolism presumably by reacting with proteins in the lipid phase of membranes, and that the chemically-treated fat cells do not have chemically modified glucose transport molecules or insulin receptors, although these proteins have altered activity. Thus, plasma membrane proteins other than the glucose carrier and the insulin receptor may be involved in insulin action.

M-PM-Po102 PHYSIOLOGICAL CONCENTRATIONS OF INSULIN STIMULATE $^{22}Na^+$ EFFLUX FROM FROG SKELETAL MUSCLE. J. O. Baker, J. C. Hansen, E. A. Hedden, D. G. Brunder and R. D. Moore, Biophysics Laboratory, SUNY Plattsburgh, NY 12901.

Previous work from this laboratory (Moore, J. Physiol. 232:23, 1981) has established that 250 mU/ml insulin stimulates active Na^+ efflux from frog skeletal muscle. To further investigate the physiological relevance of this effect of insulin, our earlier studies of $^{22}Na^+$ efflux from frog sartorius have been repeated using lower (100 and 500 μU/ml) concentrations of insulin in the presence of 0.1% bovine serum albumin (BSA). Extensive screenings of BSA were performed until a preparation was obtained that itself had no effect on $^{22}Na^+$ efflux. Charcoal-purified (Chen, J. Biol. Chem. 242:173, 1967) globulin-free BSA and a preparation obtained from M. P. Czech were found to have the least effect upon $^{22}Na^+$ efflux. In the presence of these purified albumin preparations, 100 μU/ml insulin stimulated $^{22}Na^+$ efflux by 5.3 to 18.2% ($P<0.005$) and 500 μU/ml insulin stimulated $^{22}Na^+$ efflux by 11.0 to 31.9% ($P<0.005$). In all cases, the maximal insulin effect was observed within 50 minutes after addition of hormone. The size of these effects is similar to those obtained previously (Moore, 1973) using much higher concentrations of insulin the absence of albumin. The demonstration of the stimulation of Na^+ efflux by these low concentrations of insulin supports the view that these effects are of physiological relevance. This work was supported by NIH Grants AM21059 and AM17531.

M-PM-Po103 THE EFFECT OF INSULIN ON [^{3}H]OUABAIN BINDING UNDER EQUILIBRIUM CONDITIONS. **J.C. Hansen and R.D. Moore, Biophysics Laboratory, S.U.N.Y., Plattsburgh, NY 12901.**

In order to further define the mechanism whereby insulin stimulates active Na^+ efflux from frog skeletal muscle (Moore, J. Physiol. 232:23, 1973), the effect of insulin on [^{3}H]ouabain binding to frog sartorius was determined under conditions in which ouabain binding had reached equilibrium. [^{3}H]ouabain binding was measured essentially according to the method outlined in Venosa and Horowicz, J. Memb. Biol. 59:225, 1981. Paired sartorius muscles were exposed to Ringer containing 1.2×10^{-5} M total ouabain (26-33 μCi/ml [^{3}H]ouabain) for times ranging from 30-360 minutes. Binding occurred at constant temperature ranging from 22-25°C. When insulin was used, 250mU/ml was the final hormone concentration. The muscles were then washed out through glycoside free Ringer at 1.0-1.5°C for a total of 330 minutes. The amount of ouabain remaining in the muscle versus time was fitted to a sum of exponentials, using a least squares computer program, and the intercept of the slowest component at t = 0 used to give the amount of [^{3}H]ouabain specifically bound to pump sites (Venosa and Horowicz, 1981). At a ouabain concentration of 1.2×10^{-5} M, half maximal binding occurs within 20-25 minutes, although between 3-4 hours of exposure is required before binding equilibrium is reached. Based on these results, the effect of insulin on ouabain binding was determined at 6 hrs. of exposure. The amount of bound ouabain per μm^2 membrane of insulin treated muscles was not significantly different from the amount bound by their paired controls. This data fails to support the view that insulin is increasing the number of Na pumps as has been previously reported (Erlij and Grinstein, J. Physiol. 259:13, 1976) to explain the effect of insulin on active Na^+ efflux. **This work was supported by NIH Grants AM21059 and AM17531.**

M-PM-Po104 ERYTHROCYTE MEMBRANE PROTECTIVE DRUGS PENETRATE IN SUBSECONDS TO SECONDS INTERVALS. Rex Lovrien and Richard A. Anderson, Biochemistry Department, University of Minnesota, St. Paul.

Several kinds of lipid, anesthetic, and drug molecules are striking in their ability to protect the human erythrocyte against hypoosmotic shock as P. Seeman showed (Pharmac. Rev. 24 (1972). Most such experiments are quasiequilibria experiments, relying on phenomena observed after periods of a few hundred seconds and still continuing when quenched. How rapidly can such compounds actually penetrate the membrane to do their work, regardless of the interpretation put on the quasiequilibrium experiments (Franks and Lieb, Nature 292 (1981)? We used a 'shotgun' double syringe apparatus (Biophys. J. 20 181-191 (1977)) to compare two classes of experiments: (i) Shock of cells preequilibriated with drugs; (ii) Crossover experiments, in which the shocking solution and drug are introduced simultaneously, before the restoring syringe fires subseconds to a few seconds later. The erythrocytes "see" and must bind the protective drug at the same time the cells are being osmotically shocked in the class (ii) (crossover) experiments. The principal conclusion is that numbers of such compounds penetrate the membrane in 1-5 seconds or less, at 25°. Estimates of lesser times can be roughly calculated from $\Delta\chi^2 = 2Dt$. The results probably are not dependent simply on free diffusion through the membrane, but on later reactions after the compound diffuses in.

M-PM-Po105 Ca^{++}-INDUCED LYSIS OF PLATELET SECRETORY GRANULES. J. Van der Meulen and S. Grinstein
The Hospital for Sick Children, Toronto, Ontario, Canada.

Platelet secretory granules were used for in vitro studies bearing on the mechanism of exocytosis. A morphologically homogeneous preparation of α-granules was obtained from porcine platelets. The granules were shown to be osmotically fragile, as determined by electron microscopy, turbidity measurements and the release of protein upon hypotonic lysis. Lysis could also be induced in isotonic media by addition of gramicidin to granules suspended in NaCl or KCl. Increasing the osmotic strength of the medium prevented this effect. The antibiotic had no effect when added to granules in sucrose or choline chloride solutions.

Submillimolar concentrations of Ca^{2+} drastically reduced the turbidity of granule suspensions while releasing protein to the supernatant. Electron micrographs showed that Ca^{++}-treated granules lost most of their electron-dense core, indicating that lysis had occurred. Mg^{2+} and other divalent cations failed to induce this response. The effect occurred in NaCl or KCl but not in sucrose or sorbitol solutions. Increasing the osmotic content of the medium with sucrose suppressed the Ca^{2+}-induced lysis. It is suggested that Ca^{2+} increases the ionic permeability of the granule membrane leading to solute influx followed by osmotically coupled water uptake and therefore swelling and lysis. During platelet activation, Ca^{2+}-induced granule swelling could provide the driving force for membrane fusion leading to exocytosis. Supported by M.R.C. (Canada).

M-PM-Po106 CATION INTERACTIONS WITH MEMBRANES AND EXTRACTED LIPOPOLYSACCHARIDE FROM ESCHERICHIA COLI. Richard T. Coughlin, A. Haug, and Estelle J. McGroarty, Michigan State University, East Lansing, MI 48824.

The levels of various metal cations bound to extracted lipopolysaccharide (lps) and to the intact outer and cytoplasmic membranes from Escherichia coli k12 were analyzed by inductively coupled plasma emission spectroscopy. All three isolates were found to contain significant levels of Mg, Ca, Na, Fe, Al, K, and Zn (in order of decreasing amounts in the outer membrane). The relative ratios of the various metal cations bound to membranes and extracted lps were significantly affected by the growth medium. The outer membrane contained substantially more magnesium and calcium than did the cytoplasmic membrane, but the levels of the other bound ions were similar. The ratios of metal cations bound to extracted lps reflected the levels bound to the intact outer membrane. Electrodialysis of lps removed one mole of magnesium per mole of lps without significantly affecting the levels of the other multivalent cations. Dialysis of lps against Na-EDTA removed bound Mg, Ca, and Zn, but the levels of Fe and Al were unaffected.

Using the electron spin resonance probes 5-doxyl stearate and 4-dodecyldimethyl ammonium-1-oxyl-2,2,6,6-tetramethyl piperidine we showed that the physical properties of several salt forms of lps were dramatically different. Headgroup and acyl chain mobility decreased significantly upon addition of divalent cations to the sodium salt of lps. Titration of sodium lps with divalent cations revealed discrete divalent cation binding sites.

M-PM-Po107 THE ISOLATED PORCINE THYROID CELL MEMBRANE - A NUCLEAR MAGNETIC RESONANCE STUDY. G.L. Jendrasiak. (Intr. by H. Bank). The Citadel and Medical University of South Carolina, Charleston, SC 29409 USA.

Porcine thyroid cell membranes have been isolated and studied by nuclear magnetic resonance (NMR) techniques. For comparison purposes, NMR studies of porcine erythrocyte membranes were also made. The thyroid membrane NMR spectra were quite similar to the spectra for the erythrocyte membranes except for one difference in the hydrocarbon chain-methyl group region. The reason for this difference is as yet unexplained. The effect of sonication and temperature were also studied. There appears to be a difference in "fluidity" between the two membranes. No evidence for an iodide interaction was obtained although such an interaction was observed for phosphatidylcholine, a major membrane component. The presence of phosphatidylethanolamine in the membrane may be responsible for this absence. Possible modification of the membrane lipids, so as to affect iodide transport, is discussed.

M-PM-Po108 A FLUORESCENT NINHYDRIN-POSITIVE LIPID FROM THE CYTOPLASMIC MEMBRANE OF GREEN PHOTOSYNTHETIC BACTERIA. J. M. Olson, E. K. Shaw,* J. S. Gaffney,* Biology Department and Department of Energy and Environment, Brookhaven National Laboratory, Upton, NY 11973, and C. J. Scandella*, Biochemistry Department, State University of New York, Stony Brook, NY 11794.

A new lipid comprising 10-20% of the total membrane lipid of Chlorobium limicola f. thiosulfatophilum has been purified by HPLC and TLC and then hydrolyzed in 6N HCl at 110°C for 5 hr to yield three products. One product is a fatty acid (C14:0); the other two products are water soluble fluorescent compounds. Compound A is ninhydrin positive with a fluorescence emission maximum about 390 nm and excitation maxima at 220, 292, and 332 nm at pH 3.3. Compound B (ninhydrin negative) has an emission maximum about 400 nm and excitation maxima at 220, 265, 304, 330, and 350 nm at pH 3.0. The intact lipid in hexane (6), 2-propanol (8), and water (1.1) has one emission maximum at 340 nm and excitation peaks at 232, 285, and 300-325 nm. Compound A in the same solvent also has one emission maximum at 340 nm and excitation peaks at 237, 288, and ~326 nm. The fluorescent chromophore in the intact lipid appears to be related to compound A, which may be an aromatic heterocyclic substance such as a pteridine or pterin. The lipid contains no phosphorus, no glycerol, and no ester bonds; the fatty acid appears to be linked to compound A by an amide link. This novel lipid has properties suited for binding bacteriochlorophyll a-protein (a water-soluble protein) to the inside of the cytoplasmic membrane so as to form the characteristic baseplate for attaching the chlorosome. (Supported by U.S. Department of Energy.)

M-PM-Po109 STUDIES ON THE CHARGE-ASSOCIATED MEMBRANE SURFACE PROPERTIES OF RAT YOUNG AND OLD RED BLOOD CELLS BY PARTITIONING. Harry Walter, Ali Pedram and Eugene J. Krob. Lab. of Chem. Biol., VA Med. Ctr., Long Beach, CA 90822 and Dept. of Physiol. & Biophys., Univ. of Calif., Irvine, CA 92717.

Rat erythrocytes (RBC) undergo charge-associated surface changes as a function of cell age (detectable by partitioning in aqueous dextran (D)-poly(ethylene glycol) (PEG) phase systems having an electrostatic potential between the phases) while human RBC do not (BBRC 97: 107, 1980). By combining isotope (^{59}Fe) labeling of rat RBC of different ages with countercurrent distribution (CCD) of the cells in D-PEG systems one finds that RBC of different ages have dramatically different partition coefficients, K. Young reticulocytes have the lowest K in the RBC population which increases rapidly to the highest K as these cells become young mature erythrocytes. The K subsequently diminishes over the entire life-span of the cell. To determine what surface groups are responsible for the difference in the charge-associated properties of rat young and old RBC we have subjected RBC populations containing labeled cells of different but distinct ages to CCD after various treatments. Treatment of cells with neuraminidase, or glutaraldehyde-fixation, or neuraminidase treatment followed by glutaraldehyde-fixation, or glutaraldehyde-fixation followed by neuraminidase treatment have all been found NOT to eliminate the distinctive Ks of mature RBC of different ages. Lipid extraction of glutaraldehyde-fixed red cells (with ethanol) obliterates any difference between mature RBC of different ages. (However since the latter procedure also extracts some of the isotopic label we are studying the significance of this on our results.) Neuraminidase treatment of RBC populations appears to affect reticulocytes and erythrocytes differentially: the K of mature RBC is reduced to a greater extent than is the K of reticulocytes.

M-PM-Po110 FLUORINATED ANESTHETICS AS A PROBE FOR MEMBRANE STRUCTURE. Alice M. Wyrwicz*, J.C. Schofield[+] and C. Tyler Burt**, *Dept. Chem. U. of Ill. Circle, Chicago, IL, 60680, [+]Biol. Res. Lab., U. of Ill. Med. Center, Chicago, IL, **Dept. Radiol., Harvard Med. School, Boston, MA.

We have previously shown[1] that some fluorinated anesthetics exhibit multiple environments in human erythrocytes. We now extend these observations to erythrocytes from other species and to excitable tissues such as muscle and nerve. Three different phenomena were found during the course of these studies. One, the ^{19}F chemical shifts of the anesthetics were observed for a number of different erythrocytes and were found to vary with the species. Two, the appearance of multiple populations was strongly dependent on the tissue/anesthetic interaction with muscle always showing one peak (with the anesthetics used, halothane and methoxyflurane) and nerve always showing several. Finally with nerve, the aging of the tissue led to the collapse of the several peaks to a single one.

The anesthetic can be in a rapid or slow exchange (on the NMR scale) with a membrane/tissue source depending on the anesthetic used and the composition of the membrane. In either case, a specific chemical shift is obtained which can be used as an indicator of the species and age related differences.

[1]Wyrwicz, A., Schofield, J., and Burt, C.T. (1981). Multinuclear NMR observation of intact blood, in Non-Invasive Biochemical Techniques (J. Cohen, ed.), Plenum Publishing Co., in press.

M-PM-Po111 INHIBITION OF DEHYDRATION DAMAGE TO A BIOLOGICAL MEMBRANE BY CARBOHYDRATES. John H. Crowe and Lois M. Crowe, Dept. of Zoology, Univ. of Calif., Davis, CA 95616

Water is assumed to be required for the maintenance of the structure of biological membranes (Tanford, 1980, The Hydrophobic Effect). Nevertheless, many organisms are capable of surviving complete dehydration. A common feature among such organisms is the accumulation of the disaccharide trehalose. We have used the well-known physiological and structural characteristics of sarcoplasmic reticulum (SR) to study the effects of dehydration on a biological membrane, and the effects of trehalose in inhibiting such damage. When SR vesicles which have been lyophilized in the presence of trehalose are placed in water, they show Ca transport and Ca dependent ATPase activity similar to those of fresh vesicles. Freeze fracture studies show that dry vesicles appear to be embedded in a matrix of trehalose, and rehydrated vesicles are not significantly different morphologically from fresh vesicles. The preparation must contain more than about 0.3 g trehalose/g dry wt. membranes for this effect to occur. At lower concentrations of trehalose the membranes fuse and upon rehydration form large vesicles which show reduced Ca transport and a change in the distribution of intramembrane particles. Freeze fracture of membranes dried without trehalose show lateral phase separation of protein and lipid, lipid crystals, and hexagonal$_{II}$ phospholipid. Sucrose also produces effects similar to trehalose, but greater than 1 g sucrose/g dry wt. membrane is required. Simultaneous measurement of water content and temperature changes in membranes dehydrating without trehalose show that in the region 0.2-0.3 g H_2O/g membrane a series of exothermic events occurs. When the same membranes were rehydrated from the vapor phase a series of endothermic events was recorded in the region of 0.2-0.3 g H_2O/g membrane. In membranes dried with trehalose, thermal activity was depressed. Supported by grants PCM 80-0470 from NSF and RA/41 from National Sea Grant.

M-PM-Po112 CHARACTERIZATION OF SITES OCCUPIED BY THE ANESTHETIC NITROUS OXIDE IN PROTEINS, LIPIDS AND TISSUE BY INFRARED SPECTROSCOPY. J.H. Hazzard, J.C. Gorga, O. Einarsdottir, and W.S. Caughey, Department of Biochemistry, Colorado State University, Ft. Collins, CO 80523.

The distribution of nitrous oxide molecules among aqueous and nonaqueous sites in proteins, phospholipid vesicles, red cells, and brain tissue can be directly observed by infrared spectroscopy. The frequency and shape of the N-N-O antisymmetric stretch band are dependent upon the character of the local environment around the N_2O molecule. Studies in pure solvents demonstrate (a) that stronger polar interactions between the solvent and the N_2O vibrating dipole increase band frequency (ν_3), e.g., for cyclohexane, benzene, acetone, and water ν_3 values are 2216, 2220, 2227, and 2231 cm^{-1}, respectively, and (b) that bandwidths ($\Delta\nu_{\frac{1}{2}}$) reflect the degree of uniformity of interactions of N_2O with surrounding solvent molecules, e.g., for CCl_4, $CHCl_3$, cyclohexane, and hexane $\Delta\nu_{\frac{1}{2}}$ values are 7.4, 9.3, 11.0 and 14.5 cm^{-1}, respectively. Analysis of the infrared spectrum of N_2O in a suspension of phosphatidylcholine vesicles in buffer reveals three bands. The band at highest frequency (greatest polarity) can be shown to be due to N_2O in buffer. The other two bands at lower frequencies are consistent with N_2O in highly nonpolar environments as in organic solvents. The presence of N_2O in sites less polar than water is also demonstrated in red cells and bovine and dog brain tissue as well as in purified proteins such as hemoglobin A, albumin and cytochrome c oxidase. Direct interaction of N_2O with proteins at multiple sites is clearly evident as are variations in the sites among different proteins. Fatty acid esters also reveal a difference between alkane-like and ester-like environments. These results indicate that infrared spectroscopy can provide unique information, quantitative as well as qualitative, on the distribution of anesthetics and other vibrators into protein, lipid and aqueous sites within intact tissue. (Support: U.S.P.H.S.grant#HL-15980.)

M-PM-Po113 DIFFERENCES OF LIPID-PROTEIN ASSOCIATIONS IN ERYTHROCYTES OF DUCHENNE MUSCULAR DYSTROPHY. Surendra P. Verma and Donald F. H. Wallach, Tufts- New England Medical Center, Boston,MA

We have studied Raman spectra (CH stretching) of erythrocyte membranes from Duchenne muscular dystrophy (DMD) and aged matched normal controls over the temperature range of -30° to 50°. Three prominent bands are observed around 2850 cm^{-1}, 2880 cm^{-1} and 2930 cm^{-1}. The intensity of 2880/2930 cm^{-1} decreases/increases at higher temperatures while that at 2850 cm^{-1} remains almost unchanged. The plots, $[I_{2880}/I_{2850}]$ vs temperature (T) and $[I_{2930}/I_{2850}]$ vs T reveal thermal transition of lipid or lipid-protein membrane domains and membrane proteins respectively. DMD membranes show broad transitions with onset and completion temperatures at 7±3° and -5° to -10° respectively. The protein transition starts between 25° to 30° and complete somewhere above 50°. The aged matched controls show two relatively cooperative transitions with onset/completion temperatures of 13°/21° and 2°/-12°. The transition reported by $[I_{2930}/I_{2850}]$ vs T starts near 33° and completes at 42°. In normal membranes, the midpoint of protein transition shifts from ∿ 40° at pH 7.4 to ∿ 30° at pH 6.5. In contrast DMD membranes show virtually no pH sensitivity in this range. At pH 5.7, the DMD membranes show a very broad protein transition with onset/completion temperatures 12°/42°. At pH 5.3, the protein transition disappears. Analysis of Amide I and Amide III bands indicates a greater proportion of beta-structure of DMD membrane proteins. Our data suggest a protein anomaly producing abnormal lipid-protein associations in DMD membranes.

Supported by Muscular Dystrophy Association of America, Inc., grant.

M-PM-Po114 THE INFLUENCE OF LOW-LEVEL (2450 MHz) MICROWAVES ON THE (Na^+-K^+)APTase ACTIVITY OF HUMAN ERYTHROCYTE MEMBRANES. P.D. Fisher, W.A.G. Voss, M.J. Poznansky. The University of Alberta Edmonton, Alberta, Canada, T6G 2H7.

We have reported that microwaves affect $^{24}Na^+$ efflux from human erythrocytes (1). One of the effects observed was a 25% decrease in the ovabain-sensitive portion of the $^{24}Na^+$ efflux from exposed erythrocytes incubated at 21-24°C. This effect was not observed at other temperatures. We report here on a microwave induced effect of similar temperature specificity, on the (Na^+-K^+) ATPase activity of leaky erythrocyte ghosts.

Human erythrocytes were washed then lysed in 5mM phosphate. Subsequent washing removed all detectable hemoglobin. The ghosts were then frozen and thawed twice to insure that any ghosts that may have resealed were again leaky (2). The ghosts, suspended in 100mM NaCl, 5mM $MgCl_2$ and 5mM ATP, were then exposed to microwaves for 3 hr with and without 10mMKCl. The ATPase activity of these samples were compared with those of control samples incubated at the same temperature. The exposed samples absorbed microwaves at a rate of 3mW/ml. Phosphate and protein were determined by modifications of the Fiske-Subbarow and Lowry methods respectively.

A 25-50% decrease ($P<.05$) in the (Na^+-K^+)ATPase activity of microwave-exposed ghosts incubated in the 21-24°C range was observed. This effect was not observed at other temperatures between 10° and 37°C. The implications will be discussed.

(1) Fisher, P.D., Poznansky, M.J. and Voss, W.A.G. (1981) Biophysical J. 33: 2(2), p.40A.
(2) Dunham, E.T. and Glynn, I.M. (1961) J. Physiol. 156: 274-293.

M-PM-Po115 QUANTITATIVE ANALYSIS OF Ca-PO_4 INDUCED AGGREGATION OF CYTOSKELETON-FREE ERYTHROCYTE VESICLES. K.S. Leonards & S. Ohki, Dept. of Biophysical Sciences, SUNY/Buffalo, Buffalo, NY 14214.

Large (0.5-1.0μM) cytoskeleton-free vesicles have been obtained, without hemolysis, by a new procedure from intact rabbit and human erythrocytes. Using these vesicles we have examined the roles of the membrane proteins and lipids in determining the extent and kinetics of CA-PO_4 induced erythrocyte aggregation. In addition, the effects of pH, temperature, [Ca], and $[PO_4]$ on the extent and kinetics of this process were also measured. The results indicate a linear relationship between vesicle concentration and aggregation, with both rabbit and human erythrocyte vesicles being colinear, despite their different sialic acid contents. The slope of this line was found to depend on the temperature and the length of the incubation period of vesicles with PO_4 before CA addition, but not on [Ca] or $[PO_4]$. The time dependence and saturation characteristics of the effects of PO_4 suggest a specific interaction with the membrane surface. However, enzymatic modification of the vesicle proteins with trypsin, chymotrypsin or pronase had no effect on either the extent or kinetics of aggregation. Neuriminidase treatment very slightly reduced the extent of aggregation. Combined with the above findings this suggests that vesicle aggregation does not involve the membrane proteins, but may be slightly modified by non-protein sialic acid containing molecules. Synthetic lipid vesicles composed of either egg PC or human erythrocyte outer monolayer lipids (SM, PC, PE) minus cholesteral did not aggregate under the above conditions, but some aggregation of outer monolayer lipid + cholesterol vesicles was observed, suggesting a possible involvement of cholesterol molecules in the interaction of PO_4 with the membrane surface. (Supported by NIH Grant GM-24840).

M-PM-Po116 A SPIN LABEL STUDY OF RADIATION INDUCED DAMAGE IN MEMBRANES. M.J. McCreery and C. E. Swenberg, Radiation Sciences Department and W.A. Hunt, Behavorial Science Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814. (Intr. by D.R. Livengood).

Radiation induced structural and biochemical aberrations leading to cellular dysfunction and death may be a manifestation of damage to the plasma membrane. Using nitroxide spin labels, the effects of ionizing radiation on both the lipid matrix and protein moieties have been investigated for rat erythrocytes and their isolated ghost membranes. The 5-, 12-, or 16-doxyl stearic acids and doxyl cholestane were incorporated into the membrane by dissolving the probe in chloroform, evaporating a coat of the spin label on the inside of a test tube and then incubating with the membranes for 20 minutes. The stearic acid probes exhibited no detectable changes in their spectra for membranes exposed to up to 100,000 rads ^{60}Co gamma. However, comparison of irradiated with control membranes probed with cholestane showed small but significant changes in the spectra. Membrane proteins were labelled with 4-(2-bromoacetamido)-2,2,6,6-tetramethylpiperidinooxyl and 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl by dissolving the probe in Pipes buffer at pH 6.8 and incubating with membranes for 12 hours. Free probe was removed by repeated centrifugation. The spectra of both probes revealed an immobilized and a very mobile component. For the maleimide label no difference in the spectrum between irradiated samples and control were detected. For the iodoacetamide probe the EPR signal for the irradiated sample was reduced significantly more by $NiCl_2$ than the unirradiated membranes. This enhanced reduction of signal intensity indicates that the thiol groups labelled are more exposed after irradiation. This result is consistent with the protrusions seen in electron microscopy of cells exposed to radiation. Order parameters of these spin labels and the results of saturation transfer experiments using the maleimide probe will be reported.

M-PM-Po117 ISOLATION AND CHARACTERIZATION OF LARGE (0.5-1μM) CYTOSKELETON-FREE VESICLES FROM INTACT ERYTHROCYTES. K.S. Leonards and S. Ohki, Dept. of Biophysical Sciences, SUNY at Buffalo, Buffalo, New York, 14214.

An essential prerequisite for understanding the molecular interactions which govern the biochemical and biophysical properties of membranes is the choice, or development, of a simple well-characterized, yet biologically relevant membrane model system. To this end, we have developed a procedure for obtaining large (0.5-1μM), cytoskeleton-free vesicles from intact human and rabbit erythrocytes. The vesicles are produced from erythrocytes by 'budding', using a $CaCl_2$ and EDTA 'titration' procedure. The production of vesicles occurs without hemolysis. The vesicles maintain their cytoplasmic integrity and normal membrane orientation, and are resistant to hemolysis over the pH range 5.0-11.0, and temperature range of 4-50°C. SDS-gel electrophoresis indicates that the only proteins present in the human erythrocyte vesicles are the band 3 region proteins and bands PAS-1, -2, and -3, in addition to hemoglobin and three other cytoplasmic proteins. The protein composition of the rabbit erythrocyte vesicles is similarly simple. Enzymatic treatment of the erythrocyte vesicles with trypsin and chymotrypsin indicate that all of the band 3 and glycoproteins have maintained their normal membrane orientation. The size and stability of these vesicles have made them amenable to the quantitative and kinetic analysis of membrane interactions (vesicle aggregation, etc.) using the techniques of the physical chemist. In addition, it has been possible to distinguish between membrane interactions which involve the lipids and/or proteins of the membrane proper, and those which are consequences of cyto-skeleton-membrane interactions. (Supported by NIH Grant GM-24840).

M-PM-Po118 INDEPENDENT LATERAL DIFFUSION OF THE b-c_1 COMPLEX AND CYTOCHROME OXIDASE IN THE MITOCHONDRIAL INNER MEMBRANE. M. Hoechli, L. Hoechli, and C.R. Hackenbrock. Laboratories for Cell Biology., Department of Anatomy, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514.

Double immunofluorescence microscopy was used to analyze the distribution of the b-c_1 complex (b-c_1) and cytochrome oxidase (aa_3) on both sides of the inner membrane of rat liver mitochondria. Gelatin coated glass coverslips were mounted on glass slides leaving a chamber between the coverslip and slide. Spherical mitochondrial inner membranes were attached to the coverslip in a packed monolayer. These membrane spheres were induced to fuse by addition of 10 mM Ca in 5 mM KPi, pH 6.5 creating intact membrane spheres with diameters up to 50 μm. To study the inner surface of the enlarged membranes, the whole slide mount was subjected to sonication for 1 sec which left only membrane sheets attached to the coverslip. The distribution of bc_1 and aa was determined with fluorescein-isothiocyanate and teramethylrhodamine isothiocyanate respectively, after conjugation of these fluorochromes to immunoglobulins (IgG) monospecific for the purified heme proteins. At IgG concentrations between 0.02 mg/ml and 1 mg/ml, rings of fluorescence were observed at the periphery of the intact membrane spheres, while diffuse fluorescence occurred over the inner surface of the membrane sheets. This indicates that both cytochromes occur on both membrane surfaces in a random distribution. Subsequent addition of a secondary IgG directed against either of the two primary IgG's led to the formation of separate patches of the fluorechromes. These observations indicate that bc_1 and aa_3 diffuse laterally in the plane of the membrane and do so independently of one another. Supported by NSF grant PCM 7910968 and NIH grant GM 28704 to C.R.H.

M-PM-Po119 CHRONIC EXPOSURE TO INHALED ANESTHETICS INCREASES CHOLESTEROL CONTENT IN ACHOLEPASMA LAIDLAWII by Donald D. Koblin and Howard H. Wang. Department of Biology, University of California, Santa Cruz, California 95064.

Acholeplasma laidlawii cells were grown in cholesterol-enriched medium and exposed continuously to either air (control), 4.0% halothane in air at 1 atm pressure (4% atm halothane), or 80% cyclopropane in oxygen for 24 hrs at 37°C. Cells grown in the presence of 4% atm halothane or 80% cyclopropane had approximately twice as much membrane cholesterol content per milligram of protein as the control cells. Cells grown in an anesthetic environment also tended to have a higher membrane cholesterol/phospholipid molar ratio compared to control cells. Membranes isolated from halothane-exposed cells grown in a cholesterol-enriched medium were more ordered at 37°C (measurements were made with no anesthetic present) than membranes from control cells grown in an identically enriched medium. This difference in membrane physical state between control and anesthetic-exposed cells decreased as the temperature decreased, and disappeared at approximately 23°C. Continuous exposure of A. laidlawii to 4% atm halothane or 80% cyclopropane for 24 hrs did not markedly affect membrane fatty acid composition, either in cells grown on an unsupplemented medium or in cells grown in a medium enriched in myristic, palmitic, or stearic acids. These results further support the hypothesis that an increased membrane cholesterol content may play a role in the tolerance or dependence that develops after chronic exposure to anesthetic agents.

M-PM-Po120 " Quantitation of the Forster Energy Transfer for Bidimensional Systems: A Theoretical Approach to the Study of Lateral Phase Separation, Protein Distribution and Protein Aggregation State in Biological Membranes".
Carlos Gutierrez-Merino. Depto. de Fisiología. Facultad de Medicina. Universidad de Extremadura. Badajoz. Spain.

Analytical solutions of the average rate of the Forster energy transfer for several processes affecting intrinsic membrane proteins embedded in biological membranes are presented. The analytical approach developed here is mainly based on geometrical considerations and in the fact that the average rate of energy transfer in a bidimensional system like a biological membrane is mostly determined by the contributions coming from nearest-neighbor donor-acceptor pairs. The physical phenomena analyzed are: lateral phase separation of lipid-lipid mixtures, lateral phase separation of lipid-protein mixtures (i.e. formation of eutectics), changes in the aggregation state and non-random distribution of intrinsic proteins in reconstituted systems. All of these phenomena have been repeatedly suggested to play an important role in the control of the various functions regulated or carried out by biological membranes. It will be shown that the average rate of energy transfer among protein and lipid molecules labelled with donor and acceptor molecules, respectively, allows to differenciate among these processes and also that the average rate of energy transfer can be used to quantitize these phenomena.

M-PM-Po121 OBSERVATIONS ON THE INTERACTION BETWEEN ERYTHROSINE B (RED DYE NO. 3) AND OTHER FOOD DYES ON BRAIN MEMBRANES. C. A. Lewis and R. A. Floyd, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

Food dyes such as Erythrosine B (FDC Red No. 3) have been implicated in hyperkinesis and learning disabilities in children. Experimental results with brain membranes demonstrate that the nitroxide group of membrane spin-labels such as 5-doxylstearic acid is reduced via an Erythrosine B transient species that is formed in the presence of H_2O_2 and horseradish peroxidase. Erythrosine B is more effective in mediating oxidation-reduction events occurring outside the membrane into the membrane bilayer than the other dyes, Rose Bengal, Yellow Dye No. 5, Yellow Dye No. 6 and Red Dye No. 40. Incorporation of any of these named dyes into brain membranes does not significantly alter membrane viscosity. Preliminary studies show that Rose Bengal, Red Dye No. 40, Yellow Dye No. 5, and Yellow Dye No. 6 do not behave like the Erythrosine B with the brain membranes in the presence of horseradish peroxidase and hydrogen peroxide. The results of photosensitization studies as well as oxygen consumption studies using brain membranes and the dyes show that Erythrosine B and Rose Bengal are particularly effective in enhancing the rate of O_2 consumption in the brain membranes.

M-PM-Po122 EVIDENCE FOR CHOLESTEROL BILAYER IN GASTRIC MICROSOMAL VESICLES. Tushar K. Ray, J. Nandi, Andrew Dennaman and Gerald B. Gordon. Departments of Surgery and Pathology, SUNY-Upstate Medical Center, Syracuse, NY, USA 13210.

Previous studies from this laboratory with mild ethanol (15%) treatment (60 s at 0°, 25° and 37°C) of pig (Arch. Biochem. Biophys. 202, 8-17, 1980) and rabbit (Biochem. J. 182, 637-640, 1979) gastric microsomes suggested the presence of cholesterol "patches" on microsomal surface. In the present report, digitonin was used as a tool to further investigate the topology of cholesterol. Microsomal vesicles (1 mg) were treated with digitonin (0.8 mg) for different time at 0-4°C under isotonic conditions. The effects of digitonin treatment of the vesicles on removal of cholesterol, ultrastructural changes, $(H^+ + K^+)$-ATPase activity and gastric ATPase dependent H^+ uptake ability were investigated. Microsomal cholesterol was depleted in an exponential manner with a $t_{\frac{1}{2}}$ of 12 min. There was no release of microsomal phospholipids by digitonin treatment during the same period. At 30 min of digitonin treatment visible "holes" appeared on the vesicles with a concomitant abolition of $(H^+ + K^+)$-ATPase dependent H^+ uptake. Under the same conditions the K^+-stimulated ATPase activity, however, was moderately (about 30%) reduced although the response of K^+-stimulation to valinomycin was obliterated. Prolonged digitonin treatment caused gradual diffusion and eventual disappearance of the "holes" with the generation of "cup"-shaped microsomes. The data strongly suggest free mobility of the membrane lipids and that cholesterol is present on the microsomal vesicles in the form of "bilayer patches" stabilized by the surrounding phospholipids. (Supported in part by USPHS, AM 00623 and Surgical Fund, Department of Surgery)

M-PM-Po123 THIOCYANATE, CYANATE OR NITRITE UNCOUPLES THE GASTRIC MICROSOMAL $(H^+ + K^+)$-ATPase FUNCTION. J. Nandi and T.K. Ray, Department of Surgery and Physiology, SUNY-Upstate Medical Center, Syracuse, NY, USA 13210.

Gastric microsomal vesicles enriched in $(H^+ + K^+)$-ATPase are known to accumulate H^+ in exchange for K^+. The effects of SCN^-, OCN^- and NO_2^- on vesicular H^+ transport were investigated using purified pig gastric microsomes. The vesicular accumulation of acridine orange (AO), which is a measure of H^+ uptake, shows a sigmoidal kinetics in presence of increasing K^+ with a hill coefficient of 2.27 and a S_{50} of 19.05 mM. The K_a for stimulation of the gastric ATPase by K^+ is about 1 mM. SCN^-, OCN^- and NO_2^- do not affect the microsomal $(H^+ + K^+)$-ATPase although they inhibit AO uptake in a dose dependent manner; the K_i being 5, 16 and 166 mM respectively. Addition of SCN^- at the steady-state level quickly releases AO and the effect is similar to those of the known inhibitors of $(H^+ + K^+)$-ATPase such as Zn^{+2}, F^{-1} and PCMBS. Neither SCN^- nor the inhibitors had any protonophoretic activity as demonstrated by the lack of their effects on the kinetics of spontaneous dissipation of artificial pH gradient. Studies with vesicles preequilibrated with 150 mM KCl in presence and absence of SCN^- demonstrate that SCN^- is needed within the vesicle interior to exert its inhibitory effect. The inhibition of AO uptake by SCN^- (up to 5 mM) could be reversed by an elevation of intravesicular K^+. Under all circumstances, as above, the effects of OCN^- and NO_2^- were qualitatively similar to SCN^-. The data strongly suggest that the SCN^- effect is exerted by way of interference with a low affinity K^+ site (S_{50}=19.05 mM) within the domain of the gastric ATPase complex. This low affinity K^+ site is accessible only from the vesicle interior and appears to be essential for the vectorial transport of H^+ by the microsomal $(H^+ + K^+)$-ATPase system. (AM 00623)

M-PM-Po124 THE CYTOSOL-MEMBRANE INTERFACE OF ERYTHROCYTES STUDIED BY RESONANCE ENERGY TRANSFER (RET). J. Eisinger and J. Flores, Bell Laboratories, Murray Hill, NJ 07974.

We have embedded n-(9-anthroyloxy)-stearic acid probes (n-AS) in the membranes of human erythrocytes and measured the efficiency (T) of RET from them to the cytosol hemoglobin. It is known that the probes remain in the outer phospholipid bilayer since T increases with n, the stearic acid carbon number to which the fluorophore is attached. T was obtained from the fluorescence decay kinetics of n-AS labelled intact cells and ghosts. According to a model in which the acceptors form a semi-infinite continuum beyond a plane whose normal distance to the donor is d, T is proportional to ρ/d^3, where ρ is the acceptor (heme) concentration of the cytosol-membrane interface; ρ may be estimated from the ionic strength dependence of T which increases with ionic strength as a result of cell shrinkage and was found to reach a maximal limit at about 700 mosm. Under physiological conditions ρ is found to be 14 mM, compared to an average cell heme concentration of 20 mM. From T, ρ and the Förster distances (random orientation) for the various probes one obtains the following instances from the n-AS donors to the plane of closest approach of hemoglobin: 44Å, 36Å, 32Å, 31Å for n = 2, 6, 9, and 12, respectively. For 9-vinyl anthracene donors which are presumeably between the bilayers, d = 28Å. These results show that cytosol hemoglobin comes extremely close to the inner phospholipid headgroups. When the pH is lowered[1] and hemoglobin acquires positive charge, its concentration near the membrane is even greater.

By studying the energy transfer from probes in the membrane to hemoglobin outside the intact erythrocyte it is concluded that the exterior distance of closest approach is greater than 100Å.

1. J. Eisinger, J. Flores and J. M. Salhany, PNAS (U.S.) (1982, in press).

M-PM-Po125 THE PARTIAL MOLAR VOLUMES OF N-ALKANOLS IN ERYTHROCYTE GHOSTS AND LIPID BILAYERS. Yoshiko Kita and Keith W. Miller. Depts. of Anesthesia and Pharmacology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Partial molar volumes, $\bar{V}_2$, of molecules in membranes reveal information about the packing of membrane components. Furthermore, membrane expansion by small lipophilic molecules has been hypothesized to be the basis of their anesthetic action. We have measured $\bar{V}_2$ of heptanol and octanol in red cell ghost membranes and egg phosphatidylcholine bilayers (EPC) at 25.0° C using an oscillating tube density meter. For heptanol, $\bar{V}_2$ was 142 ± 5.1 and 139 ± 2.4 ml/mole, and for octanol 159 ± 4.0 and 159 ± 1.4 ml/mole, in ghosts and EPC respectively. Thus, $\bar{V}_2$ has the same value in the lipid bilayer as it has in the biological membrane, suggesting that the alcohols pack similarly in each environment. In both cases, the $\bar{V}_2$ is essentially equal to the molar volume, V^o, of the alcohol in its own liquid. This contrasts with area changes induced by alcohols (Seeman and Roth, B.B.A. 255 190 (1972)) which are some five times greater than expected from V^o. The volume and area measurements together suggest that the membrane expands anisotropically. This could occur because either (1) lipid bilayer thickness decreases as its area expands by analogy with the situation in thermal expansion, or (2) the alcohols induce a conformational change in a protein leading to an area increase which cannot be accompanied by a volume increase. Finally, to test the critical volume hypothesis of anesthetic action, we calculated the percentage volume change of the membrane at general anesthetic concentrations. It was 0.16 ± 0.036 and 0.41 ± 0.057 percent in ghosts and EPC respectively comparable to previous predictions based on the pressure reversal of anesthesia.

M-PM-Po126 STRUCTURALLY RELATED LONG CHAIN ALCOHOLS PRODUCE DIFFERENTIAL PERTURBATIONS IN MEMBRANES AND LIPIDS OF ESCHERICHIA COLI. Andrew S. Janoff, L. Firestone and K.W. Miller, Depts. of Pharmacology and Anesthesia, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114 and E.J. McGroarty and R.T. Coughlin, Dept. of Biophysics, Michigan State University, East Lansing MI 48824.

Selective probes of membrane architecture are required for detailed investigation of membrane structure. Using spin labelling techniques, we studied the interaction of cis and trans tetradecenol with purified membranes and extracted lipids from E. coli. In cytoplasmic membranes, both isomers slightly depressed the midpoint of the broad thermotropic structural transition, T_m, but the trans isomer significantly elevated while the cis isomer slightly depressed the T_m of the extracted lipid fraction. In outer membranes, the cis isomer significantly lowered but the trans isomer slightly elevated the T_m; both isomers slightly depressed T_m in the extracted lipids. In synthetic lipids, both isomers lowered the T_m of dimyristoyl phosphatidylethanolamine but the cis isomer lowered whereas the trans isomer raised the T_m of 1-stearoyl-2-oleoyl phosphatidylcholine. In dipalmitoylphosphatidylcholine the cis isomer lowers, whereas the trans isomer has no effect upon T_m. Thus head group structure and the degree of acyl chain saturation modulate the type of lipid perturbation produced by these isomers. By comparison, the data from biomembranes clearly implicate the role of lipid-protein interactions in the observed transitions because the cis-trans differences changed upon lipid extraction. Thus cis-trans isomers of alkenols can selectively probe several components of membrane organization.

M-PM-Po127 INTERACTIONS BETWEEN ERYTHROCYTES AND MODEL MEMBRANES S. R. Bouma and W. H. Huestis
Department of Chemistry, Stanford University, Stanford, California 94305

Red blood cells incubated with model phospholipid membranes undergo lipid exchange that produces alterations in cell membrane structure. Attendant to these structure changes are at least two processes that result in appearance of cell protein in supernatant lipid aggregates. One such process is cell fragmentation, which can be induced by a variety of expedients (e.g. calcium loading or ATP depletion) and which yields lipid-protein aggregates representative of the cell membrane phospholipid content and intrinsic protein population. A second, kinetically distinct process results in selective transfer of proteins to exogenous lipid aggregates.

M-PM-Po128 X-RAY DIFFRACTION ANALYSIS OF $HgCl_2$-LABELED MYELIN REVEALS PHOSPHATIDYLETHANOLAMINE (PE) PLASMALOGEN ASYMMETRY. **D.A. Kirschner and A.L. Ganser, Department of Neuroscience, Children's Hospital Medical Center, and Dept. of Neuropathology, Harvard Medical School, Boston, MA. 02115.**

To obtain a detailed picture of the molecular organization of lipids and proteins in myelin we are correlating the structure of the heavy atom labeled intact membrane with the in vitro interactions between heavy atoms and membrane components. Depending on different conditions of salt and pH, 0.5 mM or more $HgCl_2$ induces distinctive structural changes in peripheral nerve myelin as indicated by the modified X-ray patterns recorded from treated nerves. The repeat periods for these patterns are 186Å, 173Å, 161Å, and 156Å compared to the native 176Å. Membrane electron density profiles calculated from the data to 30Å spacing show that the membrane packing is significantly altered. In order to calculate a high resolution profile from the diffraction data to 15Å spacing we searched for a single membrane structure that would best account for the different diffraction patterns. Assuming conservation of structure seems reasonable since the conditions for obtaining the different structures involve merely changes in salt composition. The most striking change after $HgCl_2$ treatment is the large increase in electron density in the lipid headgroup region in the cytoplasmic half of the bilayer. The magnitude and location of this increase suggests labeling of myelin lipid. Analysis of the lipids from $HgCl_2$-treated nerves and of extracted lipids treated with $HgCl_2$ shows that PE plasmalogen reacts with $HgCl_2$ to form a mercurialdehyde and lyso-PE. The localization of increased electron density is likely HgCl- bound to the hemiacetal intermediate of the reaction. From a correlation of our X-ray and chemical analyses we conclude that PE plasmalogen, which comprises about 80% of the PE, is preferentially localized in the cytoplasmic half of the myelin membrane bilayer. (Supported by NIH-NINCDS #14326)

M-PM-Po129 THE ORGANIZATION OF LIPOPROTEINS FROM NORMAL AND CHOLESTEROL FED RABBITS. Paulus A. Kroon, Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065.

When rabbits are fed a cholesterol enriched diet, their plasma cholesterol levels increase from an average of 60 mg/dl to over 1000 mg/dl within a month. This is accompanied by a large increase in the cholesteryl ester (CE) to triglyceride (TG) ratio in VLDL and LDL. The value of the TG/CE ratio has a profound effect on the motional state of the lipoprotein core lipids. The temperature dependence of the spectral amplitude of the lipoprotein methylene and choline methyl proton NMR resonances showed that VLDL and LDL from cholesterol fed rabbits each undergo a thermal order→ disorder transition, with midpoint temperatures of 42° and 38°C respectively. These data indicate that their core cholesteryl esters are in an ordered radial smectic phase at physiological temperatures. In contrast, no transition could be detected for the same lipoproteins from normal rabbits over the temperature range studied (20°-55°C). NMR data shows that the normal VLDL and LDL core lipids exist in a liquid state at physiological temperatures. The observation that LDL from normal chow fed rabbits does not undergo an order→ disorder transition is attributed to its high TG content, which in neat TG/CE mixtures is high enough to remove both the cholesteryl ester liquid→ cholesteric and cholesteric→ smectic transitions. In this respect LDL from normal rabbits differs from LDL from man, pigs, monkeys and dogs which all contain less TG, and undergo clear order→ disorder transitions in the range of physiological temperatures.

M-PM-Po130 IONIC COMPOUNDS WHICH ALTER HUMAN ERYTHROCYTE MEMBRANES AS SEEN WITH ELECTRON SPIN RESONANCE SPECTROSCOPY. D.L. Mazorow, A. Haug, and E.J. McGroarty. Michigan State University, E. Lansing, Michigan 48824

Fresh whole human erythrocytes were used to study the effects of ions on the erythrocyte membrane properties. Various anionic agents affect the human erythrocyte membrane structure as determined using electron spin resonance (esr) probing techniques. Membrane order parameter was increased with certain anionic agents, the degree of perturbation dependent on the anion and the anion concentration. Control studies were performed in a physiological bicarbonate buffer. N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid (HEPES) was found to be the most perturbing of the anions used with phosphate less perturbing and sulfate least perturbing. Our results suggest that anions may effect their changes of membrane order by partially or totally blocking the anion port. To substantiate this, an anion port blocking agent 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene (DIDS) was added to the erythrocytes. DIDS significantly ordered the erythrocyte membrane. Blocking the anion port may indirectly induce a change in membrane properties as seen with esr. The calcium ionophore A23187 also increased membrane order. This ionophore allows Ca^{2+} ion influx into the erythrocyte which presumably induces cross-linking of the phosphatidylserine head groups and in turn increases membrane order. (Funded in part by a General Research Support Grant from the College of Osteopathic Medicine, Michigan State University)

M-PM-Po131 HOW MUCH NON-PLANARITY IS NEEDED TO CREATE LATERAL DIFFUSION ANISOTROPY ON MEMBRANES? Boris M. Aizenbud, Chem. Dept., M.I.T., Cambridge, MA 02139, and Nahum D. Gershon, P.S.L., D.C.R.T., N.I.H., Bethesda, MD 20205

Membranes are usually assumed to be planar when diffusion coefficients are calculated from the results of fluorescence photobleaching recovery (FPR) experiments. It was shown for a model system that under typical conditions of membrane topography, and for the particular geometry of spot FPR, the calculated diffusion coefficient can be weakly sensitive to the microvilli lengths (Fed. Proc. 39:1990 (1980)). However in some experimental situations the surface topography might be more important. Smith, Clark and McConnell (Proc. Natl. Acad. Sci. U.S.A. 76:5641 (1979)) found, by two dimensional periodic pattern fluorescence photobleaching recovery, that the diffusion of succinyl-concanavelin A receptors on the surfaces of adherent mouse fibroblasts cells, having parallel stress fibers, is anisotropic. In this work we analyze quantitatively the possibility that anisotropic non-planarity of the membrane can create such an observed anisotropic diffusion on the cell surface. We considered a surface that is periodic only in one direction (e.g. perpendicular to the direction of the stress fibers). We find that the diffusion along the periodicity can be described by an effective diffusion coefficient given by $D(\lambda/L)^2$ where D is the diffusion coefficient along the surface, λ is the wavelength and L is the length of the real diffusion path contained in one period. For the value of the reported anisotropy, the amplitude of a periodic surface should be about 0.4 times the wavelength. We discuss the requirement of such a relation to the cell surface topography.

M-PM-Po132 ATTACK BY UREA OF THE APICAL SURFACE OF AMPHIBIAN URINARY BLADDER. INFLUENCE ON ANTIDIURETIC RESPONSE. M. Svelto, M. Tauc, R.M. Hays, and J. Bourguet. Département de Biologie, C.E.N. Saclay, France.

It is now well established that the increase in water permeability induced by antidiuretic hormone (ADH) in sensitive epithelia is accompanied by specific alterations in the fine structure of the epithelial cells. During ADH challenge, typical aggregates of intramembrane particles appear in the luminal membrane and although their exact role has not yet been completely elucidated there are strong indications that these particles represent transmembrane proteins that contain channels for water. An obvious approach is to take advantage of the transient insertion of these proteins in the apical membrane for their labelling, attack or extraction. We now report on the possibility to inhibit subsequent antidiuretic response by exposing the apical border of frog urinary bladder to low concentrations of urea, a relatively mild procedure previously used by others to extract cell surface proteins. Isolated urinary bladders from frogs (Rana Esculenta) were mounted between lucite chambers and the net water flow measured with a volumetric technique. The hydrosmotic response to maximal concentrations of oxytocin was measured before and after exposing, for one hour, the apical border of resting or challenged hemibladders to urea treatment. The results showed that exposure to urea of ADH treated bladders elicited a subsequent dose-dependent inhibition of the hydrosmotic response (60.2±12.4%,n=4 at 200 mM versus 92.4±3.2%,n=10 at 500mM). The reversibility was inversely related to the degree of inhibition (77.5±8.7%, n=4 at 200mM, versus 52.5±7.1%,n=10 at 500mM). In the resting hemibladders, no inhibition was observed at 200 & 300mM, whereas at 500mM there was a 95.3±2.1%, n=10 inhibition with a reversal of 24.6±6.8%,n=10. Basal net water flow remained unaffected in all conditions. These results thus point to a relatively restricted and specific attack, preferentially observed when the apical border is in an excited state.

M-PM-Po133 BUMETANIDE AND BENZMETANIDE BINDING TO MEMBRANES FROM SHARK RECTAL GLAND AND CANINE KIDNEY. Bliss Forbush, III and H. Clive Palfrey, Intro. by Steen Dissing, Departments of Physiology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Neutral, coupled Na^+,K^+,Cl^- cotransport in a variety of cell types is inhibited by "loop" diuretics, including furosemide, bumetanide, and benzmetanide (3-benzylamino 4-phenoxy 5-sulfamoylbenzoic acid). We have synthesized [^{3}H]-bumetanide (BU) and [^{3}H]-benzmetanide (BZ) by NaB^3H_4 reduction of the Schiff base formed from the parent amine and butyraldehyde or benzaldehyde. The products (1.6 Ci/mmole) were purified by HPLC. The cotransport system is thought to be found on the basolateral membrane in shark rectal gland, but on the apical membrane in mammalian kidney. Binding to epithelial membranes was studied using filtration on cellulose ester filters. Rectal gland microsomes enriched in the basolateral membrane marker Na,K-ATPase exhibited saturable binding of BZ in the presence of Na and KCl, but not in salt-free media. In different preparations, we found 20-45 nmole sites/mg protein with a Kd of 1-3 μM. BU was found to bind with 4-10 fold lower affinity. Binding of BZ was unaffected by 0.25 mM 3-(4-methoxy) benzylamino 4-phenoxy 5-sulfamoylbenzoic acid, a closely related but inactive analog. These results suggest that *in vitro* binding may correspond to *in vivo* transport inhibitory sites. The heavy microsomal fraction from renal outer medulla bound both BZ and BU with an affinity of about 2 μM (5-20 pmoles/mg). On sucrose gradient centrifugation, a peak of binding activity (60 pmoles/mg) was found at 36% (w/v) sucrose, partially separated from the peak of Na,K-ATPase activity at 39% sucrose. Similar results were obtained with membranes from canine renal cortex. Thus BU and BZ appear promising as tools for further characterization and isolation of the Na,K,Cl transport system. (Supported by NIH grant GM-27920).

M-PM-Po134 OLIGODENDROCYTE PROCESS ELONGATION: A HYPOTHESIS. S. Szuchet. Dept. of Neurology, Div. of Biological Sciences, University of Chicago, Chicago, Illinois 60637.

During myelination, oligodendrocytes synthesize several times their own mass of membranes per day. They also maintain these membranes (myelin) for a life-lasting time. Thus, oligodendrocytes afford a unique model not only for studying biosynthesis of membranes but also for learning about their maintenance at long distances from the cell bodies. When oligodendrocytes, isolated from young brains, are placed in culture, they align themselves in rows or groups not unlike interfascicular oligodendrocytes in vivo. The cells are small and round and extend long, thin processes. Examination by phase-contrast microscopy reveals phase-dark structures on the surface of the cells and in their processes. These structures are concentrated at the tips of the processes and at process-branching points where they form protuberances. Under the scanning beam they appear as "snow balls". del Rio Hortega (1928) drew attention to the presence of granular structures (gliosomes) in oligodendrocytes and their processes, they were particularly evident at the time of active myelination. He believed that these granules contained myelin precursors. The structures we observe are reminiscent of del Rio Hortega's granules. The membrane flow hypothesis postulates that biogenesis of membrane takes place by a transfer of membrane components from one cellular compartment to another. This transfer may be accomplished via secretory, condensing or carrier vesicles. Based on our structural observations, I postulate that: a) the granular structures we observe in oligodendrocytes and their extensions are morphological correlates to the membrane flow hypothesis; b) oligodendrocyte processes elongate by the translocation of these structures and c) oligodendrocyte-oligodendrocyte interaction may play an inductive role on the appearance of these granules.

M-PM-Po135 IN VIVO AND IN VITRO FT-IR STUDIES OF *ACHOLEPLASMA LAIDLAWII B*.

D.G.Cameron, H.L.Casal, A.Martin and H.H.Mantsch.

National Research Council of Canada, Ottawa,Ontario, K1A 0R6, Canada.

Temperature-induced changes in the state of the membrane lipids of *Acholeplasma laidlawii B* have been studied using Fourier transform infrared spectroscopy. Membranes were labeled biosynthetically by growing the organism in a medium supplemented with specifically deuterated fatty acids. By varying the conditions of growth such as growth temperature, the nature of the deuterated fatty acids, and the presence or absence of avidin, the effect of these parameters on the lipid phase behavior were studied. The lipid components of the membranes were separated and their phase behavior was compared with that of the whole membranes. This demonstrated that membrane proteins do not determine to a great extent the lipid phase behavior in this organism.Isolated lyophilized membranes were studied, as well as whole cells and the live organism. The thermotropic behavior of membrane lipids in intact viable cells shows dramatic differences with respect to the thermal response of isolated membranes.

M-PM-Po136 EFFECTS OF ANESTHETIC GASES ON BIOMEMBRANE PHASE DIAGRAMS, COMPRESSIBILITY AND FUSION: A THEORETICAL STUDY. Timothy J. O'Leary, Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20205.

The effects of small nonpolar anesthetic gases on biomembrane compressibility, phase diagrams, and fusion are explored from a theoretical perspective. We generalize a previously proposed (O'Leary, Biophysical Chemistry, in press) statistical mechanical model of impurity-containing biomembranes to include nonzero lateral spreading pressures. Using this model, which is based on Nagle's model (Nagle, J.Chem. Phys. 63, 1255, 1975) we show that anesthetic gases monotonically decrease the compressibility of lipid monolayers and bilayers constrained to a fixed surface pressure. When the membrane area, rather than pressure, is held fixed, the membrane may pass from a two-phase region to a single phase region. This is accompanied by an increase, then a decrease, in the compressibility of the expanded phase. We show that these compressibility changes may be expected to affect the rate of fusion of adjacent lipid bilayer membranes, through the use of a transition state model in which the formation of point defects in each bilayer is required for fusion to occur. In this transition state formulation, the probability of fusion of a fixed surface pressure vesicle to a fixed surface area membrane has a small increase, then a larger decrease, as the concentration of anesthetic is increased. This situation mimics that found at the presynaptic nerve terminal, and may be the explanation for the biphasic changes seen in clinical anesthesia.

M-PM-Po137 INVESTIGATIONS OF THE SURFACE COMPONENTS OF THE SINDBIS VIRUS. M. E. Burr, E. A. Dawidowicz, B. Sears and L. Neuringer, Francis Bitter National Magnet Laboratory, M.I.T. and Biophysical Laboratory, Harvard Medical School.

We have used the R. communis agglutinin (RCA) and a purified phosphatidyl choline (PC) specific exchange protein (PLEP) from beef liver to study the topology of the outer surface of the Sindbis virus. RCA quantitatively agglutinates the intact virus. This process was reversed by the addition of lactose to the agglutinated virus. We have shown that this agglutination of the Sindbis virus is due to the interaction of RCA with the viral glycoproteins, using a model membrane formed by reconstituting the isolated viral glycoproteins with PC. The phenomenon of viral agglutination enabled us to separate Sindbis virus from PC vesicles in a rapid and reproducible manner. Using this separation procedure after incubations with the PLEP, we have found that a significant fraction of the biosynthetically incorporated [^{3}H-choline] PC can be exchanged from the outer surface of the Sindbis virus to a PC acceptor vesicle. This study provides an approach for investigating the effect of lipid composition in the outer surface of the virion on the glycoprotein mediated lectin agglutinability in the Sindbis virus. Supported by NIH grants GM-22175 and GM-25689.

M-PM-Po138 FUSION OF SINDBIS VIRUS WITH PHOSPHOLIPID MODEL MEMBRANES. M. E. Burr, D. C. Gibbes, B. Sears, E. A. Dawidowicz and L. Neuringer. Francis Bitter National Magnet Laboratory, M.I.T. and Biophysical Laboratory, Harvard Medical School.

The initial event in viral infection involves the interaction of the viral surface with the target cell. In order to understand the molecular basis for these events, we have investigated the the interaction of Sindbis virus with model membranes which represent the target cell. We have shown that Sinbis virus exhibits a pH dependent fusion with unsonicated dispersions of phospholipids composed of egg phosphatidyl choline: egg phosphatidyl ethanolamine and cholesterol (1.0:1.0:1.5 molar ratio). Fusion was observed at pH 6.0 but does not occur at pH 7.2. We have examined the effect of sonicating the lipid dispersion on this fusion process. After sonication, there is no longer any tendency of the phospholipid vesicles to fuse with the Sindbis virus at any pH. This latter finding could result from possible effects of sonication: due to either the change in the radius of curvature of the vesicle or the disproportionation of phosphatidyl ethanolamine onto the inner vesicle surface. Supported by NIH grants GM-22175 and GM-25689.

M-PM-Po139 POLYAMINE FACILITATION OF MEMBRANE FUSION Keelung Hong and Demetrios Papahadjopoulos, Cancer Research Institute, University of California, San Francisco, CA 94143

The aliphatic polyamines--putrescine, spermidine and spermine--are natural constituents of most living organisms. Most of the effects exerted by polyamines on biological membranes cannot be ascribed entirely to the polybasic nature of these compounds. We have found that less than 100μM spermine is capable of inducing fusion of phospholipid vesicles containing both phosphatic acid and phosphatidylethanolamine. With 1mM Mg^{2+} present in the vesicle, suspension less than 50μM spermine is sufficient to induce membrane fusion at a rapid rate. Higher concentration of spermidine (500μM) is required for the fusion at the same rate as spermine-induced fusion. These are the cases that do not require Ca^{2+} for membrane fusion. For Ca^{2+}-induced membrane fusion in general, polyamines enhance the rate of fusion to a different extent for each polyamine. The significance of polyamines' effect on biological membranes is discussed.

M-PM-Po140 PHOTOPOLYMERIZATION OF VESICLES. Patricia N. Tyminski, Encarnacion Lopez, David F. O'Brien and Thomas H. Whitesides, Eastman Kodak Company, Research Laboratories, Rochester, NY 14650.

Molecules analogous to biological and synthetic lipids have been prepared which contain conjugated diacetylene moieties in the long alkyl chain. These lipid diacetylenes form bilayer structures when suspended in aqueous buffers. Ultraviolet light exposure initiates the polymerization of the diacetylenes in the lipid bilayer. The photopolymerization of diacetylenes proceeds as a 1,4-addition reaction to give a fully conjugated structure which is highly colored. The reaction is topotactic and its efficiency depends on the correct alignment of the monomeric units. Vesicles of lipid diacetylenes are photopolymerizable if the hydrocarbon chains are in a regular lattice found at temperatures below the lipid transition temperature; polymerization is inhibited above this transition. Lipid diacetylenes analogous to phosphatidylcholine (1), to a dialkyl dimethylammonium (2) salt, and to a dialkyl phosphate (3) have been prepared. The conformational preference of phosphatidylcholines in bilayer membranes indicates the two fatty acid chains extend unequal distances into the bilayer. In contrast, the synthetic lipid molecules have a plane of symmetry which suggests both chains penetrate equally into the bilayer. The photopolymerization of the symmetrical synthetic species 2 and 3 is dramatically more efficient than that of 1. Based on estimates of photoproduct extinction coefficients, the quantum yield for photopolymerization of 2 in hydrated bilayers is $\sim 10^3$ times as great as for the phosphatidylcholine diacetylene (1) bilayers. The photoreactivity of compound 3 is comparable to that of 2. These remarkable differences are interpretable in terms of the expected conformational preference of the lipid molecules.

M-PM-Po141 CAVEATS AGAINST THE USE OF THERMODYNAMICALLY UNSTABLE VESICLES AS MODELS FOR BIOLOGICAL SYSTEMS. E.A.Evans, Univ. British Columbia, Vancouver, B.C., N.L.Gershfeld, N.I.H., Bethesda, Md., L.Ginsberg, Middlesex Hosp. Med. Sch., London, U.K., and V.A.Parsegian, N.I.H., Bethesda, Md.

When prepared by energy-consuming processes such as sonication, solvent removal, or expulsion from a pipette, Small Unilamellar phospholipid Vesicles (SUVs) are thermodynamically unstable. Several consequences result from this instability: (a) Even though the vesicles are in mechanical equilibrium, small vesicles are significantly stressed by the process of preparation. Since the bilayer thickness cannot be neglected compared to radii of curvature, one immediately expects SUVs to suffer mechanical stresses that are not symmetrically distributed across the bilayer shell. Such inhomogeneous stress should create peculiar conditions for the incorporation of proteins or small molecules. This fact makes small vesicles a potentially misleading model system for studying protein-lipid interactions. (b) The decay of an inherently unstable system reflects fusion and aggregation of stressed vesicles rather than processes characteristic of biological membranes. Indeed, laser light scattering (courtesy of R.J.Nossal) and microscopic observations of sonicated DOPC preparations, initially centrifuged to select a uniform vesicle size and optically clear for more than ten days, show that initially homogeneous SUV suspensions degenerate unpredictably, within hours or days after sonication, into mixed populations that can include very large ($\gtrsim$1μm) particles even with absolutely no bacterial contamination and small degrees ($\lesssim$1%) of lipid hydrolysis. (c) The common practice of treating the (only partially recoverable) heats (Q) of cooling and heating of unstable suspensions as though they were thermodynamic enthalpies (H) is thermodynamically illegal. It is a misleading way to infer the packing and order of hydrocarbon chains at "phase transitions" .

M-PM-Po142 CONTROLLED AGGREGATION OF GIANT PC:PS VESICLES IN CALCIUM. E. Evans and R. Kwok, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada

Micromanipulation is used to control aggregation of two giant ($> 10^{-3}$ cm dia) vesicles made from mixtures of egg yolk lecithin (PC) and phosphatidylserine (PC:PS range from 10:1 to 1:1). Two vesicles are selected and transferred from a chamber which contains 10-100 mM NaCl to a separate chamber which contains a slightly higher NaCl concentration plus Ca^{++} in the range of 0-10 mM. The higher salt content in the second chamber is used to reduce membrane tensions and/or to produce excess membrane area (over that of a sphere of equivalent volume). The vesicles are manuevered into position for contact by micromanipulators and allowed to adhere. The Ca^{++} mediated affinity for formation of contact is quantitated by the free energy reduction per unit area of contact. The affinity is determined by measurements of the vesicle membrane tensions with micropipet suction, observation of the contact zone and vesicle diameters. Even for vesicles which are initially made flaccid, tensions are induced by the contact formation and represent the opposition to enlargement of the contact zone. The size of the contact zone may be either reduced or enlarged by the alteration of the vesicle tensions ("stiffnesses"). The induced tensions range from the order of 10^{-3} to 1 dyn/cm where vesicles break. Hence, the affinities range up to ergs/cm^2. Strong adhesion occurs even for a PC:PS ratio of 10:1 in 10 mM Ca^{++} provided that the vesicles are made flaccid before the aggregation stage (i.e. the initial membrane tensions are small). (Supported in part by MRC of Canada grant 7477.)

M-PM-Po143 EFFECTS OF DEFORMABILITY ON LIPID VESICLE AGGREGATION AND FUSION. E. Evans, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, and V.A. Parsegian, N.I.H., Bethesda, Md. 20205

Stable aggregation of two vesicles is promoted by the affinity between vesicle outer surfaces but opposed by vesicle rigidity. The free energy reduction, γ, in formation of a unit area of contact, A_c, is the affinity which can range from 10^{-2} erg/cm^2 for colloidal forces up to ~ergs/cm^2 for Ca^{++} crossbridging between PS surfaces. Rigidity is recognized as the work of vesicle deformation W_d. At stable contact, $dW_d/dA_c=\gamma$ gives mechanical equilibrium; and $\gamma \cdot A_c - W_d = k \cdot T$ insures that thermal motion will not disrupt contact. Vesicle rigidity involves both intrinsic (membrane) and extrinsic (size and shape) deformability. For example, Small Unilamellar Vesicles ($\lesssim$400 Å dia) made with saturated PC are expected to become very deformable when cooled sufficiently below the transition temperature, T_m, characteristic of planar membranes. Below T_m, SUVs are only partially frozen with regional separation of liquid and crystalline chains. In contrast, Large Unilamellar Vesicles ($>$400-500 Å dia) become very stiff below T_m, because they can freeze into rigid surfaces. Heating above T_m relieves membrane stress in LUVs but increases stress in SUVs. Adhesion of spherical vesicles induces membrane stress because either the membrane must expand or the volume must decrease. Thus, a hydrostatic pressure difference will persist across the membrane for salt containing vesicles but will transiently decay for salt-free systems. Partial dehydration of LUVs reduces the initial stress whereas the initial stress increases for SUVs. The threshold for contact formation is established by the membrane bending rigidity. For this reason, SUVs normally have a higher contact threshold than LUVs unless made partially frozen as noted previously. For very large vesicles, contact is limited primarily by membrane tension. Affinity-induced membrane stresses (tension and bending) may promote the subsequent breakage and "fusion".

M-PM-Po144 PROTEIN-LIPID INTERACTIONS IN MODEL MEMBRANES. D. A. Pink*, A. Georgallas*, T. Lookman*, D. Chapman† and M. J. Zuckermann§. *Theoretical Physics Institute, St. Francis Xavier University, Antigonish, N. S. Canada. †Royal Free Hospital School of Medicine, University of London, U. K. §Physics Department, McGill University, Montreal, P. Q., Canada.

We shall present a model whereby one can understand a variety of phenomena observed in lipid bilayers containing integral proteins. It is an extension of one used before (e.g. Caillé et al. Can. J. Phys. 58 581 (1980)) combined with a lattice model of proteins in lipid bilayers (Pink, Georgallas and Chapman, Biochemistry, in press). The model contains 3 parameters related to (a) the interaction between proteins and the lipid acyl chains, (b) changes in the effective lateral pressure on lipid acyl chains due to perturbations of the polar region by the proteins, and (c) direct or indirect protein-protein interactions. The parameter (a) can be determined by ^{2}H NMR measurements. We shall present results obtained by mean field approximations and Monte Carlo simulations. By considering single isolated proteins we shall illustrate how the lateral diffusion of proteins such as cytochrome oxidase can differ from that of glycophorin, and show how measurements on the latter using DSC or NMR can be understood. We shall calculate phase diagrams for various integral proteins and show how quantities such as specific heats, ^{2}H NMR order parameters, transition enthalpies and changes in the peak positions of infrared bands can depend upon the parameters (b) and (c).

Work supported by NSERC of Canada and the Wellcome Trust, U. K.

M-PM-Po145 AGGREGATION OF DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES. M. Wong and T.E. Thompson, University of Virginia School of Medicine, Charlottesville, VA 22908.

Quasi-elastic and 90° light scattering were used to study the aggregation of dipalmitoylphosphatidylcholine vesicles in the gel phase as a function of vesicle concentration, temperature and size. Increased vesicle concentration did not appreciably change aggregate size, but did change the total number of aggregates in a manner consistent with a bimolecular collisional mechanism for the conversion of aggregates to fused vesicles. Increased temperature decreased aggregation, indicating that the disaggregation rate constant increased faster than the aggregation rate constant. As a function of size, aggregation rate decreased slightly from small to 700 Å diameter vesicles, and increased considerably for 950 Å diameter vesicles. A model is presented in which aggregation precedes fusion, and collision between aggregates triggers fusion. [Supported by N.I.H. Grants GM-23573, GM-14628 and GM-07463]

M-PM-Po146 TERBIUM UPTAKE BY LIPID VESICLES DURING TERBIUM-INDUCED FUSION

David A. Lloyd, Arnold Wishnia, and Charles S. Springer, Jr. Departments of Chemistry; Hofstra University; Hempstead, New York 11550; and State University of New York at Stony Brook; Stony Brook, New York 11794.

Small unilamellar vesicles (SUV), with membranes composed of phosphatidyl choline (PC) and phosphatidyl serine (PS) in a 1:1 molar ratio, were prepared with solutions of 100 mM chelidamic acid (CA), buffered to pH=9.8, trapped inside. These were suspended in a saline solution buffered to pH=7.4. Fusion of these SUV was induced by the introduction of 400μM Tb^{3+}, a fluorescent Ca^{2+} surrogate. Fluorescence of Tb^{3+} appeared only after fusion occurred. The shapes of the emission peaks revealed clearly that the fluorescence was sensitized by CA only in a basic environment of pH=9.8. Thus fusogenic Tb^{3+} ions are incorporated into the fused vesicle via a non-leaky mechanism.

M-PM-Po147 FUNCTIONAL MEMBRANE ASYMMETRIES INDUCED BY PULSES OF ELECTROCHEMICAL POTENTIAL GRADIENTS.
J.C.VINCENT,J.M.VALLETON and E.SELEGNY (intr.by J.HASELGROVE), E.R.A. 471 C.N.R.S., University of Rouen, 76130 Mt St Aignan. France .

A theoretical model for active transport, based on functional structures, has been calculated (E.Selegny and J.C.Vincent,Biophys.Chem.,12:93,1980) by introducing and maintaining a pH gradient accross a thick membrane in which two cycling enzymes with different pH-dependences have been homogeneously distributed. This model has been supported experimentally by a glucose pump built by using hexokinase and acid phosphatase immobilized in an agarose matrix (E.Selegny and J.C.Vincent,Biophys Chem.,12:107,1980), where the energy of vectorial transport has been brought by the exergonic degradation of ATP. In this work, we present a new theoretical model for a H^+ pump using two inversible reactions with a proton cycle. The functioning of the pump modifies intra-membrane pH profiles, and so, the functional structure is either self-stabilizing or self-destroying according to the ratio of both enzyme pH optima. Self-stabilization induces migration of both membrane functional zones toward the mid plane of the membrane. Thus, the system represents a good biophysical model for a thin , active-transport-membrane surrounded by its two diffusion or polarization layers. This self-stabilized functional structure, in an isotropic membrane, carries out vectorial transport of species, as for example H^+ , between two extra-membrane media even with symmetrical conditions . It can be induced by pulses of chemical (H^+) or electrical potential gradients .

M-PM-Po148 A MODEL SYSTEM FOR STUDIES OF SPECIFIC MEMBRANE INTERACTIONS. Bruce P. Babbitt, Stephen R. Grant, Larry K. West and Leaf Huang, Dept. of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

Membrane-membrane interaction/recognition mediated by specific ligand-receptor binding has been studied using as a model system the binding of liposomes, carrying fatty acid-derivatized α-bungarotoxin (αBGT) intercalated into the bilayer, with microsac membrane vesicle enriched with acetylcholine receptor (AchR) isolated from electroplax tissue of Torpedo californica. Liposomes were prepared using a modified deoxycholate-gel filtration method. Apparent binding affinities between liposome and microsac were found to increase significantly with increases in the toxin/lipid ratio, i.e. toxin surface density, of the liposome. In contrast, liposome binding to human red cell ghosts was minimal. Binding constants for both toxin and liposomal lipid to the microsacs were the same (K_d = 2.6 X 10^{-7}M) indicating that the liposome was bound as an intact structure and that lipid and toxin binding were mediated by the same molecular event. The evidence that this event was the specific interaction of the αBGT with AchR at the microsac surface was further supported by a dose-dependent inhibition of agonist-dependent Na^+ influx of the microsacs by liposome-bound toxin molecules. The potency of inhibition also showed a strong dependence on the density of toxin per liposome. Currently, the effects of liposomal fluidity upon the aforementioned binding characteristics are being studied. All results are evaluated in terms of a multivalent binding mechanism between membranes. This research is supported by NIH grant CA 24553 and a grant from Muscular Dystrophy Association.