W-Pos64 THE BASIC DRUG BINDING SITE OF HUMAN OROSOMUCOID. M.L. Friedman, J.P. Schwegmann, R.A. Magnotti, K.T. Schlueter and H.B. Halsall. Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172

Chlorpromazine (CPZ) binds to a region of the human serum glycoprotein orosomucoid (OMD) shared by numerous other ligands. The principal binding ratio is 1 mole per mole, and current data indicate that the individual binding domains overlap. Thus, the binding of proflavine and dipyridamole are competitive with cresyl violet, but not each other. CPZ, however, is competitive with proflavine. Fluorescence quenching has demonstrated that 1/3 of OMD intrinsic fluorescence was accessible to Cs^+, and that the quenching was enhanced by the presence of negative charge proximal to the quenched trp(s). Saturation of OMD with CPZ resulted in quenching of 60% of the intrinsic fluorescence (Biophys. J. 41 (1983) 268). Modification of trp with dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium bromide diminishes CPZ binding, and was reduced in the presence of CPZ. OMD modified to 1 trp equivalent per mole displayed unchanged Cs^+ quenching properties, but the intrinsic fluorescence was reduced by greater than 50%, and the peak maximum for emission was red-shifted by 10 nm. Analysis of the modified protein showed approximately equal modification in the two fragments CNBrI and CNBrII representing the entire molecule. These data expand our model of the binding domain for CPZ as containing two "buried" tryptophans (trp 25 in CNBrI, trp 122 or 160 in CNBrII) not accessible to Cs^+, and a third, solvent accessible trp (trp 122 or 160) situated close to negative charge(s). Hydrophilicity index analysis of the amino acid sequence suggests that trp 160 is the Cs^+ quenchable fluorophore.

W-Pos65 REDUCING SUBSTRATE BINDING TO MUSHROOM TYROSINASE C.A. Kanagy, Intro. by John Haselgrove, University of Pennsylvania, Philadelphia, PA 19104.

Tyrosinase, the Type III copper enzyme catalyzing the ortho hydroxylation of substituted phenols and their oxidation to ortho di ketones, has been isolated from edible mushrooms and separated into its constituent isozymes. A Cu-(CO)-Cu chromophore has been demonstrated which fluoresces with a maximum at 540 and which partially quenches tryptophan (330 nm) fluorescence. By analogy with similar spectra for the N. crassa enzyme (1), it is concluded that one or more tryptophans lie at or near the active site. The fluorescence from these tryptophans has been used to study the binding of cresol and catechol-type substrates (inhibitors) to the active site. Neither those substrates which absorb in the region of tryptophan fluorescence, i.e. p-nitrophenol and p-nitrocatechol, nor those which do not, e.g. p-OH benzoate and 3,4 dihydroxy benzoate, affect the intensity of the 330 nm fluorescence; however, hydroxamates, known inhibitors of the enzyme, cause a quenching of the tryptophan fluorescence. Curves describing the binding of benzhydroxamic acid (BHA) to the enzyme have been constructed. Under anaerobic conditions (met + deoxy) the beta isozyme appears to exhibit non-Michaelis-Menten binding. The binding of cresol and catechol-type substrates (inhibitors) was studied indirectly by observing their effects on the hydroxamate binding curves; results to date indicate qualitative as well as quantitative differences in the effects of p-OH benzoate and 3,4 dihydroxy benzoate on the BHA binding curve. (Supported by NSF grant PCM 84-0844 to J.M. Vanderkooi). (1) Kuiper, et. al., FEBS LETTERS, Vol. 111, No. 1 232-234, 1980.

W-Pos66 THEORETICAL STUDIES OF THE INTRINSIC CIRCULAR DICHROISM OF N-ALKYLATED PROTOPORPHYRINS. S. DEVARAJAN AND ROBERT W. WOODY, DEPT. OF BIOCHEMISTRY.

N-alkyl protoporphyrins have been formed in vivo by the reaction of compounds like ethylene with the heme of cytochrome P-450 (Kunze et al., J. Biol. Chem. 258, 4204 (1983)) and in vitro by the reaction of compounds like ethyl hydrazine with the heme of catalase (Ortiz de Montellano and Kerr, J. Biol. Chem. 258, 10558 (1983)). The modified protoporphyrin formed in such a reaction exhibits optical activity. The chirality of such N-alkylporphyrins can provide clues to the stereochemistry of the heme binding pocket and the mechanism of cytochrome P-450. We are calculating the CD of such N-alkyl porphyrins using π-MO theory to treat this inherently chiral chromophore. The basic geometry of the nonplanar N-alkyl porphyrins is derived from that of Fe(II) N-methyl tetraphenyl porphyrin (Anderson et al., Inorg. Chem. 19, 2101 (1980)). The calculated CD is sensitive to the location of the alkylated ring relative to the vinyl substituents, as observed. It is also sensitive to the deviations of the vinyl groups from the plane of the pyrrole rings to which they are attached. The calculated results will be compared with available experimental data. (Supported by USPH GM 22994.)

W-Pos67 **EXCITATION WAVELENGTH DEPENDENCE OF TRYPTOPHAN FLUORESCENCE DECAY IN PROTEINS.** Iain Johnson, Dan Harris, Tony Ruggiero, Bruce Hudson, Chien-Kao Wang and Herbert Cheung. Department of Chemistry, University of Oregon, Eugene, OR 97403 and Department of Biomathematics, University of Alabama, Birmingham, ALA 35294.

Multiexponential fluorescence decay in single tryptophan proteins has several putative origins including environmental relaxation around the excited state and the existence of conformationally distinct ground state species with different absorption spectra. As a potential means of discriminating between these processes, we have examined the excitation wavelength dependence of tryptophan fluorescence decay in rabbit muscle troponin I, phospholipase A2 and ACTH in various complexation states. Marked shifts in the temporal distribution of troponin I trp. fluorescence illustrate the type of behaviour observed. On changing excitation wavelength from 300nm to 295nm, a large proportion of the third (~7ns) component fluorescence intensity in the triple exponential decay shifts into the second (~4ns) component with essentially no change in the lifetime parameters. The propagation of these effects into the corresponding fluorescence anisotropy decays is also discussed.

W-Pos68 **NUMERICAL METHODS IN FLUORESCENCE DECONVOLUTION OF SINGLE TRYPTOPHAN PROTEIN DATA CONTAINING SYSTEMATIC ERRORS: A COMPARISON BETWEEN THE METHOD OF MOMENTS AND LEAST SQUARES.** Dan Harris, Iain Johnson, Tony Ruggiero, Bruce Hudson, C-K Wang and Herb Cheung. Dept. of Chem., U. of Oregon, Eugene, Or. 97405 and Dept. of Biomathematics, U. of Alabama, AL. 35294

Data analysis is presented for mixtures of single lifetime component standards as well as single tryptophan proteins which illustrate the relative ability of the Method of Moments (Isenberg et. al.) and of least squares to resolve multiexponential lifetime data of varying complexity.

Several cases will be presented to illustrate the relative abilities of the two methods to resolve closely spaced lifetime components under conditions where scatter is present in the protein data. It is shown that data analysis of such cases requires N-exponential components for the Method of Moments a comparable analysis may be extracted from least squares via an N+1 component fit. The sensitivity of least squares to such systematic errors is high and the ability to accurately represent a small amount of scattering via an exponential illuminating.

W-Pos69 THEORY OF THE CIRCULAR DICHROISM OF LARGE PARTICLES. D. Keller, C. Bustamente, M. F. Maestre, Dept. of Chemistry, University of New Mexico, and Lawrence Berkeley Laboratory, Berkeley, Ca.

A theory of the CD of large particles has been developed and is currently being used to investigate the origin of several anomalies observed in the CD spectra of large biological aggregates. These anomalies include differential scattering tails and so-called psi-type spectra. Previous theories in the literature have been applicable only to particles small vs. wavelength of the incident light. Our approach is similar to that of DeVoe's (1) theory, but includes the effect of multiple scattering in the particle and does not make use of dipole approximation. From an understanding of the way in which long-range chiral order affects the CD spectrum, it may be possible to use CD anomalies to get information about the structure of the large aggregates.

(1) H. DeVoe, J. Chem. Phys. vol 41, pp 893-400 (1964).

W-Pos70 FORMYCIN TRISPHOSPHATOTERBIUM(III): A FLUORESCENCE ENERGY TRANSFER COMPLEX TO PROBE PHOSPHORYL TRANSFER. William R. Kirk, Department of Biophysics, Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

A 1:1 complex of the fluorescent pyrazolopyrimidine nucleotide antibiotic Formycin A trisphosphate with Terbium(III) has been prepared and characterized. The complex displays a dramatic (10^4) sensitization of Tb+++ luminescence by energy transfer from Formycin (excited at 307 nm., Tb+++ bands observed and measured at 488, 543, and ca. 585 nm.) The Forster theory in the very weak coupling case allows a determination of the apparent distance from the electronic orbitals of Formycin to those of Tb+++ of 6.6± .6 A° (R_o of 3.4± .4 A°). The complex has a dissociation constant of 2.3± 1.3 x 10^{-7} M. (MES buffer). Approximately 4 waters remain liganded to Tb+++ in the complex, as determined by the change in quantum yield upon substitution of H_2O with D_2O (at the same pL). The complex thus has useful properties to investigate the mechanism of phosphoryl transfer with respect to metal-nucleotide enzyme interactions. (Supported by N.I.H. Grant GM25432).

W-Pos71 PROTEIN HYDRODYNAMICS AND DYE BINDING GEOMETRY OBTAINED VIA GLOBAL ANALYSIS OF MULTIPLE FLUORESCENCE ANISOTROPY DECAY EXPERIMENTS. Joseph M. Beechem, Jay R. Knutson, and Ludwig Brand. The Johns Hopkins University, Baltimore, Maryland 21218.

It is well know that excitation of multiple absorption bands in a bound fluorophore can be used to select the angles between the oscillators of a small dye and the principle diffusion axes of the larger protein molecule. The actual rotational correlation time(s) will be independent of excitation wavelength, while the pre-exponential factors may change. With a program for simultaneous analysis of multiple anisotropy decay experiments ("global analysis"), one may extend this concept to several different dyes on a single protein. Each individual experiment reflects a particular dye oscillator orientation, sampling the Brownian rotations of the body for a particular range of times (a few dye lifetimes). At each temperature, the rotational correlation time(s) will be independent of the dye utilized. Since, in this type of analysis, the total intensity lifetime differences between dyes are completely uncoupled from the determination of the rotational correlation times, these experiments can be combined together in a single analysis. The main advantage of this technique is that the principle diffusion tensor of a protein may be greatly overdetermined. For anisotropic rotors, the recovered pre-exponential factors and associated correlation times can help determine the geometry of dye binding. The polarized decay data may originate from different detectors (i.e. "T-format" without matching) or even different instrumental methods (pulsed or phase/modulation). Application of this analysis to the binding of various dyes to proteins such as horse liver alcohol dehydrogenase will be discussed.

W-Pos72 TIME-RESOLVED FLUORESCENCE STUDIES OF TYROSINE, TYROSINE ANALOGS, AND TYROSYL RESIDUES IN POLYPEPTIDE HORMONES. W.R. Laws(1), J.B.A. Ross(2), J.C. Sutherland(1), P.G. Katsoyannis(2), A. Buku(2), and H.R. Wyssbrod(2); (1) Biology Dept., Brookhaven National Laboratory, Upton, NY 11973 and (2) Depts. of Biochemistry, Physiology and Biophysics, and Center for Polypeptide and Membrane Research, Mt. Sinai School of Medicine, New York, NY 10029.

Fluorescence decays were obtained at 5° C with excitation/emission at 284/302 nm using a single-photon counting fluorimeter at the National Synchrotron Light Source. Phenol, anisol, p-cresol, and tyramine exhibit invariant single exponential decays from pH 2-7. 3-(p-OH-phenyl)-propionic acid (PPA) shows a two-state, ground-state mechanism in this pH range, with quenching by the protonated carboxyl group. Tyrosine, O-methyltyrosine, N-acetyltyrosine, tyrosylglycine, and glycyltyrosine display complex decay kinetics over this pH range which can be explained as a combination of two processes: first, titration of the carboxyl group (as in PPA); and second, different lifetimes for the three rotameric populations of the phenol ring about the $C^{\alpha} - C^{\beta}$ bond. Between pH 2-7, tyrosinamide and N-acetyl-tyrosinamide exhibit multi-exponential decays with pH independent parameters, explainable by the rotamer model: the pre-exponential terms agree with ^{1}H-NMR determined rotamer populations for the phenol ring in these compounds, allowing assignment of lifetimes to specific ground-state rotamer populations. Studies on the single tyrosyl residue in the polypeptide hormone oxytocin (and oxytocin analogs) show that the nearby disulfide bridge is a static quencher. Furthermore, the complex decay kinetics seen for the single tyrosine are consistent with the NMR determined rotamer populations. The complex decay of the four tyrosyl residues in insulin differs between monomer and dimer, indicating that insulin self-association perturbs tyrosyl environments.

W-Pos73 A RAMAN STUDY OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE BOUND TO LIVER ALCOHOL DEHYDROGENASE

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We report the first Raman spectrum of reduced nicotinamide adenine dinucleotide (NADH) when bound to an enzymatic active site, that of liver alcohol dehydrogenase (LADH). This was obtained by subtracting the Raman spectrum of LADH from that of the binary LADH/NADH complex. There are significant changes in the spectrum of bound NADH as compared to that in solution. The data indicate that both the nicotinamide and the adenine moiety are involved in the binding. At least one of the two NH_2 moieties of NADH also participates.

W-Pos74 THE DECAY AND THE FORMATION TIMES OF M_{412} IN THE BACTERIORHODOPSIN PHOTOCYCLE ARE INDEPENDENT OF THE CHROMOPHORE STRUCTURE

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Flash photolysis studies of the bacteriorhodopsin have shown that the decay and the formation of the M_{412} intermediate are both biphasic (Ort and Parsons, J. Biol. Chem. 1978, 253, 6158). We have done a systematic resonance Raman study of these various forms of the M_{412} under conditions that would allow one or the other form to predominate. The concentrations of the contributing species to the Raman spectra were obtained from the parallel flash photolysis studies. Raman spectrum of each species was then calculated by computer subtraction.

Resonance Raman spectrum is very sensitive to the chromophore structure, and we detect no difference in the Raman spectra of the various M_{412} species. We, therefore, conclude that the chromophore in these pigments has identical structure, and the differences, thus, lie in the protein structure.

W-Pos75 PURIFICATION OF THE DICARBOXYLATE CARRIER FROM RAT LIVER MITOCHONDRIA.
Ronald S. Kaplan and Peter L. Pedersen, Dept. of Biological Chemistry, The Johns Hopkins Medical School, Baltimore, MD 21205.

The mitochondrial dicarboxylate carrier has been substantially purified from rat liver mitoplasts by extraction with Triton X-114 in the presence of cardiolipin followed by chromatography on hydroxylapatite (HA). Upon incorporation of the HA eluate into phospholipid vesicles, an n-butylmalonate-sensitive malonate/malate exchange has been demonstrated. This exchange activity is enhanced 222-fold relative to the starting material (i.e. detergent solubilized mitoplasts). Silver stained SDS gels verify the high purity of this fraction relative to the starting material. Nonetheless, the banding pattern indicates that several protein species are still present.

As isolated, the dicarboxylate transporter activity is rather unstable, but can be stabilized either by storage in 10% ethylene glycol at -20°C or by incorporation into phospholipid vesicles in the presence of 4 mM malate followed by freezing in liquid nitrogen. Such proteoliposomes catalyze a malonate uptake which is characterized by a first order rate constant of 1.02 min^{-1} and a $t_{1/2}$ of 41 sec. Furthermore, malonate uptake is dependent on internal malate and can be inhibited by n-butylmalonate, mersalyl, PCMB, and Pi, but not by NEM. It is concluded that this highly purified fraction contains an active dicarboxylate transporter which exhibits a substrate specificity and inhibitor sensitivity similar to that found in intact rat liver mitochondria. Supported by NSF grant PCM 8300772.

W-Pos76 INTERACTIONS OF THE 82 kDa MITOCHONDRIAL K+/H+ ANTIPORTER WITH DICYCLOHEXYLCARBODIIMIDE (DCCD). William H. Martin, Daniel J. DiResta and Keith D. Garlid, Dept. of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

Under appropriate conditions, DCCD inhibits K+ efflux from respiring mitochondria. This effect is shown to result from DCCD inhibition of the endogenous K+/H+ antiporter, confirming the findings of a previous study (Martin, W.H., Beavis, A. D. and Garlid, K.D. [1984] J. Biol. Chem. 259, 2062-2065) in which selective binding of [14C]DCCD to membrane proteins enabled us to identify an 82 kDa protein responsible for K+/H+ antiport in rat liver mitochondria.

Inhibition of K+/H+ antiport by DCCD in Mg++-depleted mitochondria follows pseudo-first order kinetics, and the half-time for inhibition at 0°C is about 10 min using 50 nmol/mg DCCD. The rate constant for inhibition is approximately the same as the rate constant for [14C]DCCD binding to the 82 kDa protein, confirming the identification of this porter. Rat liver mitochondria contain about 7.5 pmol/mg of K+/H+ antiporter.

The rate of DCCD inhibition of the K+/H+ antiporter is decreased and the 82 kDa protein is protected from [14C]DCCD labeling by quinine, endogenous matrix Mg++, low pH and increases in medium osmolality. We find that submitochondrial particles, rat heart mitochondria and *E. coli* vesicles all contain an 82 kDa, [14C]DCCD-binding protein which is protected from radiolabeling by Mg++ and quinine. The finding in *E. coli*, which is not yet verified by transport studies, suggests that the K+/H+ antiporter may be highly conserved in chemiosmotic systems. (Supported by USPHS grant GM31086).

W-Pos77 CALCIUM BUFFERING CHARACTERISTICS AND PHOSPHOLIPASE ACTIVITIES OF RAT BRAIN MITOCHONDRIA.
G. Fiskum, D.R. Pfeiffer, K.M. Broekemeier and B. Baroody, The George Wash. Univ. School of Medicine, Wash., D.C. 20037 and the Hormel Institute, Univ. of Minnesota, Austin, MN 55912

Rat brain mitochondria (RBM) were tested for their ability to buffer the free [Ca^{2+}] of a medium over a broad range of total Ca^{2+}. The phospholipase (PL) activities of these mitochondria were also determined since it has been suggested that activation of PLA2 can limit the capacity for mitochondria to accumulate Ca^{2+}. The RBM used in these studies were isolated by a new procedure where the inclusion of digitonin was substituted for Ficoll-gradient centrifugation. These mitochondria exhibited excellent respiratory control and rates of respiration that are approximately 50% greater than values obtained with RBM isolated in the absence of digitonin. In addition, these RBM accumulated extremely large quantities of exogenous Ca^{2+}. In the presence of malate, glutamate, phosphate, and Mg^{2+}, the RBM sequestered 700 nmoles $Ca^{2+}mg^{-1}$ protein in the absence of ATP and 3500 nmoles mg^{-1} in the presence of ATP. RBM buffered the medium free [Ca^{2+}] at approximately 0.6μM in the absence of Na^+ and at 1.1μM in the presence of 20mM Na^+; however, Na^+ had no effect on the maximum capacity for Ca^{2+} accumulation. Measurements of phospholipase activities indicated that the total PL of RBM was much lower than that of rat liver mitochondria. Although the PLA1 activities were similar, Ca^{2+}-activated PLA2 activity of RBM was less than 20% of that obtained with liver mitochondria. These findings may account for the large capacity of RBM to accumulate Ca^{2+} and could relate to the response of brain mitochondria to certain pathological conditions, e.g. ischemia and post-ischemic reperfusion. (supported by an AHA Grant 84-01 to G.F. and a USPHS Grant HL-08214 to D.R.P.)

W-Pos78 THE RANGE OF MITOCHONDRIAL Ca IN SMOOTH MUSCLE. M. Bond, A.J. Wasserman, D. Kowarski, A.V. Somlyo and A.P. Somlyo. Penn. Muscle Inst., U. of Penn. Sch. of Med., Phila., PA

The range of total mitochondrial (mito) Ca concentrations *in situ* was measured by electron probe microanalysis (EPMA) in freeze-dried cryosections of vascular smooth muscle (Rabbit portal vein, PV and main pulmonary artery, MPA) rapidly frozen in the following conditions: a) relaxed, b) maximally contracted c) Na-loaded d) in Ca-free solution. Mito Ca of PV in Ca-free solution ($0Ca^{2+}$, 11-16mM Mg^{2+}, 30 min, 37°) was 0.1±0.2 SEM mmol Ca/kg mito dry wt (N=75), significantly lower ($P < 0.01$) than in PV Na-loaded at 37° (cyto [Na]=1145, cyto [K]=9mmol/kg dry wt): 1.3±0.2 (N=68); relaxed PV (37°): 1.6±0.2 SEM (N=50); contracted PV (K+NE, 30 min. 37°): 2.3±0.4 SEM (N=56); relaxed MPA (Rb-Krebs, 37°): 2.7±0.3 SEM (N=76); or contracted MPA (Rb-Krebs, NE, 37°): 2.2±0.3 SEM (N=79). In saponin-skinned PV at pCa5 (1), mitochondria Ca-load to 222±17 SEM (N=32) mmol/kg mito dry wt (1). Thus, in normal smooth muscle, mito [Ca] is of the same order of magnitude as measured by EPMA in skeletal muscle (2), retina (3) or liver (4). The significantly lower mito [Ca] in Ca-free PV than in resting PV or MPA demonstrates that mito [Ca] is variable, and can be altered over a small range of 0-2mmol/kg mito dry wt. This is consistent with the view that small changes in mito matrix Ca^{2+} regulate key mito enzymes (5), and supports the conclusion (6) that mitos do not play a major role in regulating cytoplasmic Ca^{2+}. REF.: 1) Somlyo, A.P. *et al.*, Fed. Proc. 41: 2883, 1982; 2) A.V. Somlyo *et al.* J. Cell Biol. 90, 577, 1981; 3) Somlyo, A.P. & Walz, B., J. Physiol. (Lond.) 358: 183, 1985; 4) A.P. Somlyo *et al.*, these Proc.; 5) Denton, R.M. & McCormack, J.G., FEBS LETT. 119, 1, 1980; 6) A.P. Somlyo, Nature 309: 516, 1984. Supp. by HL15835 to the Penn. Muscle Inst., MDA to MB, S.E. Penna. AHA to AJW, NHLBI Training grant HL07499.

W-Pos79 LOSS OF MAXIMAL CAPACITIES FOR Ca^{2+} ACCUMULATION AND OXIDATIVE PHOSPHORYLATION BY RAT BRAIN MITOCHONDRIA DURING CEREBRAL ISCHEMIA. F. Hamud and G. Fiskum, The George Washington University School of Medicine, Washington, D.C. 20037

Mitochondrial degradation and elevated levels of intracellular Ca^{2+} are implicated in the pathophysiology of irreversible cell damage that can occur during cerebral ischemia. In this study, free plus synaptosomal mitochondria (RBM) were isolated from control and decapitation-ischemia rat brains and tested for their capacity to respire and accumulate Ca^{2+}. The respiratory control ratio of the RBM declined from a control value of greater than 12 to values of 8, 7, 4 and 4 for 15, 30, 45 and 60 minutes of ischemia respectively. This was due to a decrease in the ADP-stimulated rates of respiration rather than an increase in the rate of State 4 respiration. The maximum capacity for RBM to accumulate and retain Ca^{2+} in a cytosol-like medium also declined significantly during 15-60 min of ischemia. In the absence or presence of ATP, the capacity to sequester Ca^{2+} and maintain a constant extramitochondrial "buffer point" decreased by 50% after 15 min and by 75% after 45 min of ischemia. The decline in the respiratory and Ca^{2+} uptake capacities of RBM may be due in part to the intracellular acidification that occurs during ischemia. *In vitro* experiments with normal RBM indicated that there is an 80% reduction in the amount of Ca^{2+} that can be accumulated when the pH of the medium is lowered from 7.0 to 6.0. These results suggest that mitochondrial degradation may influence the post-ischemic intracellular Ca^{2+} homeostasis of the brain both indirectly via the lowered capacity to rapidly produce ATP and directly via a loss in the ability to sequester abnormally large amounts of Ca^{2+} (Supported by an AHA Grant 84-01 to G.F.)

W-Pos80 RELATIONSHIPS BETWEEN Ca^{2+} RELEASE, Ca^{2+} CYCLING, AND Ca^{2+} MEDIATED PERMEABILITY CHANGES IN MITOCHONDRIA. William W. Riley, Jr. and Douglas R. Pfeiffer (Intr. by Gregory D. Reinhart), The Hormel Institute, University of Minnesota, Austin, MN 55912.

Recent reports suggest that in liver mitochondria, permeability changes accompanying Ca^{2+} release induced by t-butylhydroperoxide (tBH) are secondary events rather than causally related to release of the cation. According to this hypothesis, permeability changes result from persistent cycling of Ca^{2+} between distinct uptake and release carriers (e.g. Bellomo et al. (1984) Eur. J. Biochem. 140, 1-6) or from extramitochondrial phospholipase A_2 (PLA_2) activated by the released cation (e.g. Harris and Heffron (1982) Arch. Biochem. Biophys. 218, 531-539). To test these proposals, Ca^{2+} release induced by tBH or NEM was compared to Mg^{2+} release in the absence or presence of EGTA and/or ruthenium red (RuRed). In the absence of cycling inhibitors, Ca^{2+} release develops somewhat faster than Mg^{2+} release consistent with (but not requiring) a portion of Ca^{2+} being released by a specific carrier. In the case of tBH, EGTA and/or RuRed produce a 1:1 relationship between the extent of release of Ca^{2+} and Mg^{2+}, in contradiction to the secondary event hypothesis. In the case of NEM, RuRed also produces a 1:1 relationship while EGTA causes persistence of Ca^{2+} release in excess of what can be accounted for by the permeability mechanism. RuRed together with EGTA results in all Ca^{2+} release being accounted for by the permeability pathway. The findings indicate that in the absence of cycling inhibitors or in the presence of EGTA alone, about 65% of the Ca^{2+} released from NEM treated mitochondria crosses the inner membrane by reverse action of the uptake uniporter. A PLA_2 dependent, H^+ conductance increase with subsequent collapse of $\Delta\mu_i$ may allow operation of this efflux pathway. (Supp. by HL 08214).

W-Pos81 INHIBITION OF MITOCHONDRIAL CA^{2+} EFFLUX BY CN^-. K.K. Gunter and T.E. Gunter, Dept. of Rad. Biol. and Biophys., Univ. of Rochester, Rochester, NY 14642

We have found that Na^+-independent Ca^{2+} efflux from liver mitochondria may be strongly inhibited by addition of the metabolic blocker cyanide. This inhibition can be observed under conditions where only small amounts of Ca^{2+} (0-40 nmoles/mg above endogenous levels) are taken up by the mitochondria and where the mitochondria are protected from undergoing the permeability transformation upon addition of cyanide by use of an agent, such as Mn^{2+}, which, like Mg^{2+}, protects the mitochondria against transformation.

In a characteristic experiment, Mitochondria (1 mg/ml) were suspended in two samples in 195 mM mannitol, 65 mM sucrose, 6 mM HEPES (pH 7.2), 2mM $MgCl_2$, 3 mM KAc, 5 μM TPP, and 3 mM succinate. 20 μM Mn^{2+} was added 30 sec. after addition of 10 nmoles labelled Ca^{2+}/mg protein. $\Delta\psi$ was monitored using a TPP electrode. Ruthenium red (5 nmoles/mg) was added 8 min. after Ca^{2+} to induce unidirectional efflux in both samples. One min. later, 1 mM KCN was added to one sample. Aliquots of each sample were periodically filtered, and prepared for β counting. The results consistently indicated slower Ca^{2+} efflux in the cyanide blocked sample (0.11 nmoles/mg/min) than in the energized sample (0.38 nmoles/mg/min).

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W-Pos82 MALIC ENZYME ACTIVITY, PYRIDINE NUCLEOTIDE OXIDATION AND CALCIUM RETENTION BY AS30-D HEPATOMA MITOCHONDRIA. R.M. Risoldi and G. Fiskum, The George Washington Univ. School of Medicine, Washington, D.C. 20037

Rat liver mitochondria (RLM) will release Ca^{2+} in the presence of succinate and rotenone when their pyridine nucleotides (PN) are oxidized by the addition of t-butyl hydroperoxide (TBH) via the glutathione peroxidase-reductase system. In contrast, the retention of Ca^{2+} by AS30-D rat hepatoma tumor mitochondria (TM) is insensitive to the addition of TBH under these conditions. Both RLM and TM will release Ca^{2+} in the presence of TBH when ascorbate and tetramethyl-p-phenylenediamine (Asc-TMPD) are used as artificial oxidizable substrates. Spectrofluorometric measurements of the PN redox state showed that TBH induced complete oxidation of PN in both TM and RLM when incubated with Asc-TMPD but only in RLM when incubated with succinate and rotenone. It was hypothesized that the lack of net PN oxidation in TM was due to the presence of malic enzyme which can rereduce NAD+ or NADP+ during the oxidation of malate generated from succinate. This was demonstrated by measurements of extramitochondrial pyruvate in suspensions of RLM and TM. TM produced 5 nmoles pyruvate $min^{-1}.mg^{-1}$ protein in the presence of succinate, rotenone and TBH. No pyruvate was observed in the presence of Asc-TMPD unless malate was added which, accordingly, also inhibited TBH-induced PN oxidation and Ca^{2+} release. No pyruvate was observed with RLM under any of these conditions since RLM do not possess malic enzyme. Thus, the presence of malic enzyme in TM may be important in maintaining a reduced PN redox state under conditions which normally promote oxidation. This, in turn, could play a role in the regulation of many PN-sensitive mitochondrial activites such as Ca^{2+} efflux. (Supported by USPHS grant CA 32946 to G.F.).

W-Pos83 THE INTERACTION OF THE POTENTIAL-SENSITIVE MOLECULAR PROBE DIS-C_3-(5) WITH PIGEON HEART MITOCHONDRIA UNDER EQUILIBRIUM AND TIME-RESOLVED CONDITIONS - B. P. Bammel, J. A. Brand, D. Evans, and J. C. Smith, Dept. Chem. and LMBS, Georgia State Univ., Atlanta, GA.

Substrate addition to diS-C_3-(5)-PHM mixtures causes inhibitor-sensitive losses in the dye fluorescence yield and alterations of the probe absorption spectrum in the 575 to 700 nm spectral region. Binding work has indicated that a substantial reduction in the maximum number of binding sites, n, occurs when succinate or glutamate is present relative to the value of n obtained with PHM inhibited with cyanide; little or no change in K_D has been observed. Using rapid mixing techniques, the time course of the diS-C_3-(5) glutamate- and ATP-dependent optical signals in the PHM preparation has been monitored. The single-exponential, glutamate-dependent signal developed on a time scale of nominally 5 seconds and appears to be inversely dependent on probe concentration at fixed PHM concentration but was independent of PHM membrane protein concentration when the probe concentration was maintained constant. The ATP-dependent signal is biphasic; the faster phase signal was a linear function of membrane concentration whereas the slower phase appears to be concentration independent. The ATP signal development is some five times faster than that of the glutamate-independent one and apparently exhibits no dependence on dye concentration over the range employed in these studies. The behavior of the time-resolved data has been tenatively interpreted as indicating the accumulation of the probe in the internal volume of the mitochondrion according to the prevailing membrane potential. The signal amplititude and rate at which the ATP-dependent cytochrome c oxidase soret band shift signal in PHM develops is markedly reduced by the addition of small amounts of diS-C_3-(5), further suggesting that the permeation of the PHM membrane by this probe is occurring via an electrophoretic process that competes with the processes responsible for the soret band shift signal for the energy available from the electrochemical gradient. Support: NIH GM 30552.

W-Pos84 RAT SPERM ARE SIMILAR TO BROWN FAT CELLS: OLIGOMYCIN DOES NOT STRONGLY INHIBIT THEIR RESPIRATION RATE. Richard A. Cardullo and Richard A. Cone, Department of Biophysics, The Johns Hopkins University, Baltimore, MD 21218.

We have previously reported that the oxygen consumption rate ($\dot{Z}O_2$) of rat sperm does not change detectably as their motility (and work output) is progressively reduced to zero by increasing the viscoelastic drag of the suspending medium (Cardullo and Cone, Biol. Repro., 30, suppl. #1). In contrast, the $\dot{Z}O_2$ of sea urchin sperm becomes markedly reduced. The surprising result that rat sperm $\dot{Z}O_2$ does not vary detectably as their work output changes led us to investigate what fraction of respiratory energy is devoted to their ATP production. Sperm were diluted 40-fold into a physiological salt solution (PSS) with no added metabolites. Under these conditions, both rat sperm and sea urchin sperm require O_2 for motility. We measured respiration rates for both motile rat sperm in PSS and also for sperm immobilized with 3% methylcellulose (MC) in PSS. The following were then added sequentially to these suspensions: an aerobic metabolite, 5mM lactate, an inhibitor of oxidative phosphorylation, 1 μM oligomycin, and finally an uncoupler of oxidative phosphorylation, 10 μM "1799". The results are as shown: $\dot{Z}O_2$ (μl $O_2/10^8$ sperm-hour) ± SD for 5 trials

			Lactate	Oligomycin	"1799"
Rat sperm	PSS	23.0 ± 2.5	33.7 ± 4.6	18.8 ± 2.6	47.9 ± 6.7
in:	3% MC	21.9 ± 3.7	34.9 ± 4.5	17.9 ± 2.5	48.7 ± 6.0

Note that in all cases rat sperm $\dot{Z}O_2$ was unaffected when their work output was reduced to zero. In most cells oligomycin strongly inhibits $\dot{Z}O_2$, but our results show that, in rat sperm, oligomycin only moderately reduced $\dot{Z}O_2$ (about 50%). This makes rat sperm somewhat like brown fat cells in which oligomycin has little effect on $\dot{Z}O_2$. Therefore, it appears that a major fraction of the respiratory energy produced in a rat sperm is consumed by processes other than ATP production. (Supported by HD16800 and GM07231).

W-Pos85 SMALL-VOLUME STOCHASTIC FREE ENERGY TRANSDUCTION IN OXIDATIVE PHOSPHORYLATION. Yi-der Chen and Hans V. Westerhoff, Lab. of Molecular Biology, NIADDK, NIH, Bethesd,MD 20205

Recently Westerhoff et. al.[1] have reviewed experimental results that show deviations from Mitchell's chemiosmotic hypothesis[2]. Briefly, the variation of phosphorylation rate is not simply determined by the variation of the transmembrane electrochemical potential difference for protons($\Delta\tilde{\mu}_H$);it depends on whether $\Delta\tilde{\mu}_H$ is altered by changing the leakage or the input (electron transfer)reaction. In addition, the electron transfer chains and ADP phosphorylation systems appear to influence each other more directly than just through $\Delta\tilde{\mu}_H$.These findings led Westerhoff et. al.[3] to propose a 'mosaic' model for proton coupling, in which the oxidative phosphorylation system is assumed to be composed of a great number of small independent coupling units connecting only one or a few electron transfer chains with one or a few H^+-ATPases.

To test this idea,we have examined a theoretical system consisting of three energy transducing cycles(an input, an output and a leakage) which exchange H^+ between a small volume and an outside reservoir. It is found that indeed the above mentioned deviations can be accounted for by this model.Thus, whenever metabolic intermediates occur in small number per metabolic coupling unit, the usual kinetic and thermodynamic principles may fail to apply even though the total number of intermediates may be macroscopic.

1. Westerhoff, H. V., Melandri, B. A., Venturoli, G. and Kell, D. B. (1984) BBA in press.
2. Mitchell, P. (1961) Nature 191, 144-148.
3. Westerhoff,H.V.,Melandri,B.A., Venturoli,G., Azzone,G.F. and kell,D.B.(1984)FEBS Lett.165,1-5.

W-Pos86 PROTON LEAKAGE FROM THE GRADIENT UNDER STATE 3 CONDITIONS. T.E. Gunter, B.D. Jensen, and K.K. Gunter, Dept. Rad. Biol. and Biophys., Univ. Rochester, Rochester, NY 14642

In the context of a theory describing the steps of the process of oxidative phosphorylation, we have defined and measured a factor that accounts for the fraction of the power that leaks from the electrochemical proton gradient before being used in a phosphorylation related process. This factor is defined as: $(1-H_L) = J_H{}^L/(J_H{}^P + J_H{}^T + J_H{}^L)$, where $J_H{}^P$ is the effective proton current flowing through the ATPase pathway, $J_H{}^T$ is the effective proton current through phosphorylation-coupled transport pathways, and $J_H{}^L$ is the effective proton current through all other pathways. The factor, H_L, can be measured using the relationship $H_L = (J_O{}^{P+L} - J_O{}^L)/J_O{}^{P+L}$, where $J_O{}^{P+L}$ is the oxygen use rate coupled to phosphorylation plus leakage (state 3 rate), and $J_O{}^L$ is the (state 4) oxygen use rate under conditions where the electrochemical proton gradient is the same as it was for the relevant state 3 conditions but in which no phosphorylation is taking place. For actual measurements, we assume that $\Delta\psi$ measured by a TPP electrode is proportional to $\Delta\mu_H$ and measure oxygen use rate under nonphosphorylating conditions in which $\Delta\psi$ has been decreased to the $\Delta\psi_{state\ 3}$ value by addition of the necessary amount of malonate to mitochondria, which are oxidizing succinate. The average value of $(1-H_L)$ measured is 11% under control conditions. The factor H_L should be used to correct oxygen use rates in P/O measurements.

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W-Pos87 A MECHANISM FOR LOCAL CHEMIOSMOSIS: CAN PHOSPHOLIPIDS FERRY PROTONS FROM REDOX CHAINS TO ATPases IN MITOCHONDRIA AND BACTERIA? Stuart McLaughlin & John Kasianowicz. Dept. of Physiology & Biophysics, HSC, SUNY Stony Brook, NY 11794.

Several groups (e.g. Guffanti & Krulwich, 1984) have evidence that "proton pumping via respiration is directly or locally connected to H^+-translocating processes such as ATP synthesis". We propose that protons are transferred from a source, such as a redox chain, to a titratable phospholipid, such as phosphatidic acid (pK of PA = 8). PA then diffuses in the plane of the membrane to the ATPase and transfers the proton directly to this molecule. The life time of a proton on a PA molecule that undergoes no reactions is $1/k_D \simeq 10^{-2}$ s, whereas the average time it takes for the protonated PA to encounter an ATPase in its two dimensional random walk is only $1/k\{ATPase\} \simeq 10^{-5}$ s ($k_D/k_R = 10^{-8}$ M; $k_R < 10^{10}$ M^{-1} s^{-1}, the diffusion limited value for the heterogeneous reaction of H^+ with PA; k is the diffusion limited rate constant in two dimensions (Hardt, 1979), about 10^{17} $cm^2 mole^{-1} s^{-1}$; and {ATPase} is the surface concentration of the ATPase, about 10^{-12} moles cm^{-2}). This simple calculation illustrates the mechanism is possible if the rate at which PA loses protons to buffers in the aqueous phase is $< 10^5$ s^{-1}. We are testing the ability of phospholipids to accept protons from a source in the membrane. Our electrical measurements on bilayers containing PA exposed to the protonophore FCCP are consistent with the hypothesis that the protons are transferred rapidly from FCCP to PA and do not appear in the aqueous phase until about 10^{-2} s have elapsed. These electrical measurements complement the spectrophotometric measurements of proton transfer on micelles by Nachliel and Gutman (1984). Supported by NSF grant PCM-8200991.

W-Pos88 NETWORK THERMODYNAMICLS IN BIOENERGETICS: SOME USEFULL NEW RESULTS AND THEIR IMPLICATIONS. D. C. Mikulecky and L. Peusner, MCV/VCU, Richmond, VA 23298-0001 and Box 380, York, Maine 03909.

Network thermodynamics (NT) analysis of reaction diffusion systems [Peusner, J. Chem. Phys. 77:5500-5507 (1982)] has unified other graphical approaches to kinetics such as the King-Altman method and its counterpart developed by T. Hill (Free Energy Transduction in Biology, Academic Press, N.Y., 1977). NT uses driving forces which arise from potentials compatible with a connected network representation of the system. Representing a system as a connected passive network is equivalent to and capable of extending Onsager's reciprocity to the entire linear domain in the new coordinate system, which is of uniformly of greater extent than obtained by Onsager using thermodynamic variables. It is also possible to show that the connected networks representing pseudo-first order systems, such as carrier transport and similar systems studied by Hill, need to contain sources. Thus, these networks only are reciprocal in the Onsager sense when the source strengths in them become small enough to be negligible ("near" equilibrium). Besides providing a diagramatic way of seeing the transition from reciprocal to nonreciprocal behavior, the network thermodynamic formulation also provides a new and powerful result (Peusner in Chemical Applications of Topology and Graph Theory, R.B. King, ed. Elsevier, 1983): the ability to define a metric which measures the "distance" between states. It can be shown that the geometric interpretation of this is equivalent to embedding Onsagers' coordinates in the higher dimensional orthogal spaces of NT.

W-Pos89 *ELECTRICAL CHARGE TRANSPORT IN PROTEINS.* *Béla Karvaly, Bioelectromagnetics Laboratory, Michigan State University, East Lansing, MI 48824-1322.*

Charge transfer, especially electron and proton transfer in, between, and by proteins is not understood in sufficient detail. Most of the evidence supporting the various transfer mechanisms proposed come from conductivity measurements carried out on protein powder samples in hydrated state. Consequently, the prevailing theoretical frameworks are dominated by concepts which are solid-state physical in nature and, fail to consider the structural features peculiar to proteins. A charge transport model based upon the specific molecular properties of proteins is presented, which utilizes the genuine idea of Libby (J. Phys. Chem. 56, 865 /1952/) that the solvent rearrangement may play an essential part in the electron transfer processes in solutions. It is proposed here that the preferential electrical charge transfer pathways in proteins are formed by the primary hydration sites, amino and carboxylic groups. Water is thought to be aligned around these polar groups in a nearly spherical fashion, while forming a rather densely packed hydrate sphere, no matter whether or not net charge is present on them. Then the motion of a charge carrier from one transfer site to an other will not bring about significant changes in the degree of orientation and in the number of water molecules adjacent to either the donor or the acceptor site and, the activation free energy for charge transfer will arise from the work required to charge up the hydration shell. This activation energy is independent of the nature of charge carrier; it predicts similar hydration dependencies for electronic and protonic charge motion. The effects of polar adsorbates, the possible interrelation between electronic and protonic conduction, charge injection into proteins (and membranes), the lack of entropic contribution to charge movement, etc. will be discussed. *(Supported by DARPA/ONR Grant 0014-82-K-0739)*

W-Pos90 THERMODYNAMICS OF THE CONVERSION OF FUMARATE TO MALATE. E. Gajewski, R. Goldberg, and D. Steckler, (Intr. by W. Yap), National Bureau of Standards, Gaithersburg, MD 20899. The thermodynamics of the conversion of aqueous fumarate to L(-)malate has been studied using both heat conduction microcalorimetry and a gas-chromatographic procedure for the measurement of equilibirum constants. The reaction was carried out using fumarase in TRIS/HCl buffer in the pH range 7.3 to 8.0. The ionic strength was varied from 0.005 to 0.64 mol kg^{-1} and the temperature from 25 to 50 °C. A combined analysis of the data leads to the following at 25 °C: $\Delta G^\circ = -3546 \pm 30$ J mol^{-1}, $\Delta H^\circ = -15.63 \pm 0.10$ kJ mol^{-1}, and $\Delta C_p^\circ = -35 \pm 6$ J $mol^{-1}K^{-1}$ for the conversion of $furmarate^{2-}$ to $malate^{2-}$. The temperature dependence of the equilibrium constant (K) is: R ln K = (3546/298.15) - (15630)[(1/298.15)-(1/T)] - 35 [(298.15/T) - 1 + ln(T/298.15)], where T is in Kelvins and R = 8.31441 J $mol^{-1}K^{-1}$.

W-Pos91 A MODEL FOR THE MECHANISM OF WATER OXIDATION IN PHOTOSYNTHESIS. T. Kambara and Govindjee, Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801 USA

Water oxidation in photosynthesis is carried out through a four-step univalent redox sequence in which Mn atoms play an essential role. We present here a new model for the mechanism of the water oxidation center (WOC) that utilizes redox-active ligand chemistry and the chemistry of Mn in two different environments to explain all known aspects of electron transfer from H_2O to Z, the electron donor to the photosystem II reaction center P680. The major features of the new model are: (1) The four functional Mn atoms are divided into two groups of two Mn each: (a) [Mn], complexes in a hydrophobic cavity in the intrinsic 34kD protein, and (b) (Mn), complexes on the hydrophilic surface of the extrinsic 33kD protein. The oxidation of H_2O is carried out by the two [Mn], and the protons are released from [Mn] to (Mn) along the hydrogen bond between their respective ligand H_2O molecules. (2) Each of the two [Mn] in the cavity binds one redox-active ligand (RAL), such as a semiquinone (e.g., catechol; alternatively, an aromatic amino acid, such as tyrosine, may carry out this function). Electron transfer occurs from the reduced RAL in [Mn] to Z^+. (3) The two (Mn) are similar to Mn^{3+} complexes in aqueous media; Cl^- stabilizes the system by being indirectly coordinated to a (Mn) as an outer sphere ligand. (Mn) stabilizes the water oxidation reactions by pulling H_2O protons away and releasing them into the lumen. In our presentation, predictions from the model will be compared with the available experimental data concerning electron transfer between the WOC and Z, the proton release pattern, the electronic structure of the WOC, the deactivation of the S states of the WOC, and the effect of Cl^- ions on O_2 evolution. The present model allows for a coherent understanding of the diverse published data.

W-Pos92 THE EFFECT OF PH ON THE CONFORMATION OF PLASTOCYANIN. J.E. Draheim, G. Anderson, and E.L. Gross, Dept. of Biochemistry, Ohio State University, Columbus, Ohio 43210.

Plastocyanin (PC) acts as a mobile electron carrier in photosynthetic electron transport between cytochrome-f and P700. We studied the effects of pH on the tertiary structure of spinach PC because: 1) since PC is localized in the interior of the thylakoid membrane, upon illumination it will experience a decrease in pH from pH7 to pH5; 2) at lower pH's there is a redox-inactive form of PC even though lowering the pH also decreases the Km of the PC-P700 interaction. The near-UV absorption, CD, and fluorescence spectra show that the PC tertiary structure changes in response to pH. Previously we have shown that the tertiary conformation of PC is sensitive to both ethylenediamine chemical modification and redox state. Lowering the pH caused a 6% increase in absorbance at 278nm for oxidized spinach PC and a 14% decrease for reduced PC. At 260nm lowering the pH caused a 10% increase in absorbance for oxidized PC and a 7% decrease for reduced PC. The ellipticity at 253nm increased 56% for oxidized spinach PC and decreased 37% for reduced PC. Lowering the pH attenuates the extent of the PC conformation change associated with changing its redox state. At pH7 there is a 56% increase in absorbance at 278nm while at pH5 there is only a 29% increase. This is confirmed with the fluorescence spectra which indicates that at pH7 there is a decrease in the relative fluorescence quantum yield upon reduction while at pH5 there is no change. Similar results were obtained with poplar and lettuce PC. These results can be explained by: 1) the presence of a redox-inactive form at low pH's which becomes active subsequent to binding with P700; 2) a change in the conformation of the redox-active form of PC such that the PC-P700 interaction in enhanced.

W-Pos93 THE EFFECT OF CHEMICAL MODIFICATION ON THE CONFORMATION OF PLASTOCYANIN. G. Anderson, D. SANDERSON, J.E. Draheim, and E.L. Gross, Dept. of Biochemistry, The Ohio State University, Columbus, Ohio 43210.

Plastocyanin (PC) acts as a mobile electron carrier in photosynthetic electron transport between cytochrome-f and P700. We have studied the effects of ethylenediamine and tetranitromethane chemical modification on the tertiary structure of PC in order to: 1) determine the effect of PC charge configuration on its interactions with its redox partners; 2) gain some insight as to the location/nature of its interaction sites. The near-UV absorption, CD, and fluorescence spectra indicate that the PC tertiary conformation is sensitive to chemical modification. Previously, we have shown that ethylenediamine modification raises the PC midpoint redox potential +40mV for doubly modified PC. However, subsequent modification does not significantly change the PC redox potential further. In addition, extensive modification with ethylenediamine abolishes the cation requirement for the PC-P700 interaction. Our preliminary results indicate that even PC modified with a single ethylenediamine has a perturbed tertiary conformation. The tetranitromethane modification has been localized to Tyr83 and does not alter the PC fluorescnce implying that Tyr83 is highly quenched in native PC. The absorption and CD spectra indicate that after modification Tyr83 exhibits two transitions in the near-UV only one of which is sensitive to the PC redox state. Upon lowering the pH, the Vmax for control PC increases while the Vmax for PC modified with tetranitromethnane decreases.

W-Pos94 COVALENT MODIFICATION OF THF PHOTOSYSTEM 2 REACTION CFNTER.
Swatantar Kumar and Joseph Warden, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180-3590

Time-resolved spectroscopic techniques, including optical flash photolysis and electron spin resonance (esr), have been used in conjunction with fluorescence yield and dye-reduction assays to monitor electron transport in Photosystem 2 (PS2) subchloroplast particles incubated with the covalent modifiers phenylglyoxal and tetranitromethane. Phenylglyoxal modified digitonin (D-10) particles from spinach (75 mM, pH 7.9, 30°C, 20 min incubation) are characterized by (1) a high initial fluorescence yield (F_i), (2) an inhibition of PS2 mediated reduction of dichlorophenolindophenol (DPIP) by diphenylcarbazide (3) an abolition of flash-induced absorption transients ($t_{1/2} \geq 2\mu s$) attributed to the primary electron donor, $P680^+$ and (4) the elimination of esr Signal 2_s and Signal 2_f. These observations suggest the critical participation of specific arginyl residues on both the oxidizing and reducing sides of Photosystem 2 and also implicate phenylglyoxal as a Quinone-site inhibitor (Golbeck and Warden, BBA in press). In contrast tetranitromethane (3 mM, pH 6.0 or 7.8, 20°C, 2 min incubation) abolishes completely fluorescence and absorption (P_{680}^+) transients and in addition eliminates DPIP reduction capacity and esr Signal 2_s and Signal 2_f. These data indicate that tetranitromethane may modify selectively tyrosyl and/or sulfhydryl residues in the locus of P_{680}•pheophytin thereby preventing charge separation. The studies reported here demonstrate that covalent modifiers, in conjunction with spectroscopic analysis, can be exploited effectively to probe and categorize inhibition sites in the reaction center of PS2. (Supported by NIH Grant 2R01 GM26133-05).

W-Pos95 REQUIREMENTS FOR BINDING TO THE Qa SITE OF THE PHOTOSYNTHETIC REACTION CENTER. M.R.Gunner J.M. Bruce, P.L. Dutton, Univ. of Penna. Phila. PA 19104

The ubiquinone-10 that is tightly bound to the Q_A site of the reaction center protein of Rps. sphaeroides can be reversibly removed and replaced with other quinones. Structural factors that determine the affinity of a molecule for the site are elucidated by comparison of the equilibrium dissociation constants (K_d) of different molecules for the site, K_ds of quinones that reconstitute Q_A dependent electron transfer reaction were measured by monitoring activity as a function of quinone concentration. The affinity of compounds that do not reconstitute activity were determined by their potency as competitive inhibitors. Conclusions include (K_ds in parentheses): (1) The UQ head group (2,3-methoxy 5,6-methyl BQ) binds weakly (10μM); addition of a single isoprene unit greatly increases the affinity (110nM), (2) Increasing the size of the ring system increases affinity e.g. benzoquinone (BQ, >50mM), napthoquinone (NQ, 10μm), anthraquinone (AQ, 100nM): (3) Only one of the quinone carbonyls is required for binding (AQ, 100nM; anthrone, 300nM; anthracene, >100μM); (4a) Substitution on the quinone ring of BQs or NQs strongly increases affinity (NQ, 10μM; 2-methyl NQ, 400nM); (4b) Addition on the adjacent ring in the beta position of NQs or AQs effects the affinity only weakly (AQ, 100nM; 2-methyl AQ, 40nM); (4c) Mono substitution on the adjacent ring in the alpha position of NQs or AQs does not influence the affinity, but a second addition strongly weakens binding (1-methyl AQ, 100nM; 1,4-methyl AQ, 75μM); (5) Orthohydroxy quinones, potent inhibitors of electron transfer bind tightly to the site but do not accept electrons. They associate and dissociate from the site on the minute time scale, while all other quinones without long tails equilibrate with the site on the ms time scale.

W-Pos96 INTERACTION OF THYLAKOID ELECTRON CARRIERS WITH PARAMAGNETIC LANTHANIDE PROBES L.A. Graham and J.C. Salerno, Dept. of Biology,Rensselaer Polytechnic Institute, Troy,NY 12181

Paramagnetic lanthanide complexes have been shown to be effective probes of the exposure of paramagnetic prosthetic groups in soluble and membrane bound proteins over distances of up to~30Å. Water soluble dysprosium chelates are particularly useful in this regard. In TSF-1 particles, the EPR signals of the bound ferredoxins are broadened and power saturation is relieved by negatively charged probes such as dysprosium-EDTA at concentrations in the 1-10 mM range while positively charged probes had little or no effect. The effects were independent of redox state, indicating that both iron sulfur centers interact with probe independently; comparison with results using soluble proteins suggests 10-15Å. Soluble spinach ferredoxin EPR signals are preferentially broadened by positively charged probes, while the flavin radical of ferredoxin-NADP reductase is preferentially broadened by negatively charged probes. This alternating series suggests the involvement of ionic interactions in the formation of complexes by the electron transfer components.

Similar broadening is observed in unstacked thylakoid preparations, suggesting that both iron sulfur centers are close to the outer surface. Positively and negatively charged dysprosium compounds both broaden cytochrome f and Rieske center spectra in solubilized cytochrome b_6f complex but we have not been able to observe this behavior in thylakoids. These results suggest that these carriers are more exposed to the inner aspect of the thylakoid membrane.

W-Pos97 RECONSTITUTION OF CAROTENOIDS INTO THE REACTION CENTERS OF RHODOPSEUDOMONAS SPHAEROIDES R26. Harry A. Frank and Barry W. Chadwick, Department of Chemistry, U-60, University of Connecticut, Storrs, CT 06268.

An attempt has been made to reconstitute several carotenoids into the photosynthetic reaction centers of the carotenoidless mutant Rhodopseudomonas sphaeroides R26. By systematically varying the length of the conjugated polyene chain and attached functional groups one is able to assess the importance of these structural features in the triplet state trapping abilities of the carotenoids. Electron spin resonance spectroscopy is used to assay carotenoid triplet state formation. A procedure for reconstituting the carotenoids and analyzing their extent of binding to the reaction center complex is described. The data indicate that efficient binding determines the extent of triplet state trapping. Also, strong binding occurs only when a polar functional group (e.g. methoxy or hydroxy) is present on the carotenoid molecule.

Work supported by NSF grant PCM-8408201.

W-Pos98 RESOLUTION OF THE SPECTRA OF PHOTOSYNTHETIC PIGMENTS BY MEANS OF FACTOR ANALYSIS. Robert T. Ross*, Angel R. Arcelay, Jill M. Collins, Craig M. Davis, Tanaji S. Desai, Michael A. Marchiarullo, Department of Biochemistry, Ohio State University, Columbus OH 43210, and Bruce K. Holmer, Department of Chemistry, University of California, Berkeley CA 94720.

Factor analysis is a multivariate linear algebraic technique which can be used to study very complex systems. Using as variables excitation and emission wavelength, we have been able to determine the number of independently absorbing and emitting components observable in photosynthetic systems, to resolve excitation and emission spectra of each component, and to estimate the relative absorbance, relative fluorescence quantum yield, and Stepanov temperature of each. We have been able to resolve the spectra of PSI and PSII in green algae and plant chloroplasts, and three spectral components in the prompt emission of cyanobacteria. Using as variables emission wavelength and magnesium ion concentration, we have resolved three components in the prompt emission of plant chloroplasts. Using as variables emission wavelength and time since illumination, we have resolved two components in the delayed emission from photosynthetic material. Conventionally, factor analysis is performed on a two dimensional data array, and thus can work easily with only two controlling parameters, such as excitation wavelength and emission wavelength, or emission wavelength and time. However, fluorescence intensity can be described as a linear function of three or even four parameters; therefore we have developed an algorithm for multidimensional factor analysis of arrays of dimensionality greater than two, and begun use of this technique for the analysis of emission from photosynthetic systems.

W-Pos99 CHLOROPHYLL-PROTEIN INTERACTIONS: A MODEL APPROACH. F. LAMARCHE, R.M. LEBLANC and A. ENGLISH*, Université du Québec à Trois-Rivières, Centre de recherche en photobiophysique, Trois-Rivières, Québec, Canada and *Chemistry dept., Concordia University, Montréal, Québec, Canada.

The interaction between chlorophyll *a* (chl *a*) and some proteins was studied by the monolayer technique. All the experiments were carried out in similar manner by depositing the insoluble pigment at the air/water interface and injecting the soluble protein in the subphase. A mechanical system of prism enables us to obtain rapidly the steady state of adsorption of protein. The interaction between the two components at the interface is monitored as a variation in surface pressure (π) and surface potential (ΔV) at constant molecular area, in presence or in absence of the protein in the subphase. The results show a strong interaction between chl *a* and cytochrome *c* (cyt *c*). This interaction is slightly different at higher ionic strength. Similar experiments were performed by substituting pheophytin a (phe *a*) for chl *a* at the interface. On the basis of these results, the interaction between phe *a* and cyt *c* appears different from the one between chl *a* and cyt *c*. It is also possible to deposit the film present at the interface on a solid substrate by the Blodgett-Langmuir technique in order to measure the absorption and the fluorescence spectra. These results are consistent with a specific interaction between the chlorophyll and protein. Some other chlorophyll-protein systems were also studied, e.g. chl *a* - stellacyanin (scy) and chl *a* - plastocyanin (pcy).

W-Pos100 THE C-H VIBRATIONAL STRETCHING BANDS OF CHLOROPHYLL IN MONO - AND MULTILAYERS - AN INFRARED STUDY. C. Chapados, Centre de recherche en photobiophysique, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada, G9A 5H7.

The C-H vibrational stretch bands of chlorophyll (Chl) in monolayers, obtained by the Langmuir-Blodgett technique, have been studied by infrared spectroscopy. Compared to a solution or to a multilayer which shows three to four bands, the spectra of Chl a or Chl b molecules in monolayers have revealed more than seven bands, which are assigned to the various CH groups in the molecule. Contrary to solutions or to multilayer samples which give featureless bands, each band of the monolayers is composed of many components which are modified when the system is perturbed either by drying or by hydration techniques. The separation between the components of the CH aliphatic bands is typical of crystalline field splitting and the modification of the intensities of these components is associated with the movement of the phytyl chain of the chlorophyll molecules. The CH aromatic stretch bands have been observed; the displacement and the variation of the intensities of these bands is associated with deformation of the porphyrin ring. The CH band of the formyl group on Chl b has also been observed. The displacement and the variation of the intensity of this band are related to the association that this group makes with the surrounding molecules and with the displacement of the porphyrin ring.

W-Pos101 ESR AND ENDOR OF BACTERIOPHEOPHYTIN a RADICALS. IMPLICATIONS FOR BACTERIOCHLOROPHYLLS IN VIVO. T. Horning, E. Fujita and J. Fajer. Brookhaven National Lab., Upton, NY 11973.

Assignments of the radicals observed on oxidation of bacterial reaction centers to dimeric bacteriochlorophylls a ($P870^+$) or b ($P960^+$) are based on comparisons of ESR and ENDOR characteristics in vivo with those observed for $BChl^+$ a and b in vitro. To ascertain whether the unpaired spin distributions of the radicals can be modified by ligation to the magnesium or hydrogen bonding to the peripheral carbonyl groups of the BChls, the ENDOR parameters of the cation of bacteriopheophytin a (BPheo) have been measured as a function of solvent and temperature. 1) In CH_2Cl_2/CH_3OH mixtures, in which the Mg is complexed and the oxygen functions of the macrocycle are hydrogen bonded by the methanol, ENDOR results in solution are comparable for BChl a^+ and BPheo a^+. The differences that are observed are predicted theoretically. 2) In "non-bonding" CH_2Cl_2 solutions, the coupling constants of BPheo a^+ are identical (2%) to those in the alcoholic solvent indicating that hydrogen bonding has little effect on the spin distribution of these cation radicals. The combination of 1) and 2) suggests therefore that ligation of the Mg of the BChls and/or hydrogen bonding by methanol in vitro or tyrosine in vivo, would not significantly alter the spin profiles of BChl a^+ or $P870^+$. 3) Changes in the coupling constants of BPheo a^+ can be induced near the freezing point of CH_2Cl_2/CH_3OH mixtures. However, only the protons of rings II and IV are affected. The effect observed is attributable to conformational changes (twisting) of the flexible saturated rings induced by the glassy matrix. Similar effects could be induced by packing and protein interactions in vivo and explain some of ESR variations observed in different photosynthetic bacteria. (Supported by the Division of Chemical Sciences, DOE.)

W-Pos102 REDOX, OPTICAL AND RADICAL PROPERTIES OF BACTERIOCHLOROPHYLL g: THEORETICAL PREDICTIONS FOR THE CHROMOPHORE OF HELIOBACTERIUM CHLORUM. L.K. Hanson and J. Fajer, Department of Applied Science, Brookhaven National Laboratory, Upton, NY 11973. J.D. Head and M.C. Zerner, Quantum Theory Project, Univ. of Florida, Gainesville, FL 32611.

The anoxygenic photosynthetic bacterium Heliobacterium chlorum contains[1] a new bacteriochlorophyll (BChl) labelled "g" with a proposed[2] structure similar to that of BChl b except for the substitution of a vinyl group for the acetyl function found on ring I in BChls b and a. Unlike organisms that contain BChl b or a, H. chlorum exhibits a low energy absorption band at 788 nm, and a reversible bleaching occurs at 798 nm on exposure to light.[3] INDO-CI calculations of the optical spectra of BChls b and g correctly predict the trends observed for the pigments in vitro. The small spectral differences found[1,2] for BChl g in vitro and in vivo suggest that H. chlorum contains a monomeric BChl in its reaction center. (Green plant chlorophylls, which are also postulated to exist as monomers in P680 and P700, similarly possess vinyl instead of acetyl groups on ring I. Acetyl groups may thus help stabilize the "special pairs" in bacteria that contain BChls a and b). Extended Hückel calculations predict that cation and anion radicals of BChl g, the presumed primary donors and acceptors of H. chlorum, would exhibit ESR and ENDOR properties similar to those of BChl b radicals in vitro. Optical spectra and redox properties are also predicted. (Work supported by the Division of Chemical Sciences, DOE, under Contract No. DE-AC02-76CH00016.)

Ref: 1) Gest, H. and Favinger, J.L. Arch. Microbiol. (1983) 136, 11. 2) Brockmann, H. and Lipinski, A. ibid. (1983) 136, 17. 3) Blankenship, R.E., Fuller, R.C. and Gest, H., private communication.

W-Pos103 Ca^{2+} INDUCED PHASE SEPARATIONS IN MIXTURES OF ZWITTERIONIC PHOSPHOLIPIDS, W.Tamura-Lis, B.A. Cunningham and L.J. Lis, Department of Physics and the Liquid Crystal Institute, Kent State University, Kent, Ohio, and E.J.Reber and J.M.Collins, Department of Physics, Marquette University, Milwaukee, WIsconsin.

Ca^{2+} solutions of low molarity (< 100 mM)added to phosphatidylcholine and phosphatidylethanolamine derived from egg yolk can cause the appearance of two lamellar phases, as seen by x-ray diffraction. In particular, EYPC produces one phase which swells to large bilayer separations characteristic of charged lipid systems, while the second phase apparently swells to the same limited extent as EYPC in water. However, palmitoyl-oleoyl-PC, the synthetic analogue to EYPC, in the presence of $CaCl_2$ exhibits only one lamellar phase which swells as a charged lipid bilayer would. Mixtures of homogeneous chain PC's in the liquid crystal state, and mixtures of different head group lipids (DOPC and DOPE) produced two phases when exposed to $CaCl_2$ We conclude that two lipid species are needed to produce phase separations in the presence of $CaCl_2$, and that these phases do not separate on the basis of the species present but as a consequence of two energy minima occurring for the mixtures as a function of separation.

W-Pos104 HYDROCARBON CHAIN PACKING MODES IN LIPIDS : EFFECTS OF ALTERED SUB-CELL DIMENSIONS AND CHAIN ROTATION ON X-RAY DIFFRACTION DATA. P.R. Maulik, M.J. Ruocco, and G.G. Shipley. Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

From single crystal structures of lipids, the lateral hydrocarbon chain (HC) packing modes have been described in terms of specific HC sub-cells [e.g. triclinic ($T_{//}$), orthorhombic ($O_{\perp}$ and $O_{\perp}'$), monoclinic ($M_{//}$), hexagonal (H)]. In order to understand the changes in HC chain packing in lipid bilayers induced by variations in temperature, hydration, ion-binding, etc., we have examined the effect on the calculated x-ray diffraction pattern of (a) systematic variations in the dimensions of the HC sub-cells, and (b) the effect of chain rotation at fixed lattice sites. For example, the $\underline{a}$ and $\underline{b}$ cell dimensions of the $O_{\perp}$ sub-cell were varied from $\underline{a}$ = 7.42 to 8.40Å and $\underline{b}$ = 4.96 to 4.85Å in six steps keeping the projected sub-cell area ($\underline{a}$ x $\underline{b}$) constant and the positions and intensities of the strong sub-cell reflections calculated. In this way, the conversion of the $O_{\perp}$ sub-cell (with either fixed chain orientations or simulated chain rotation) to the hexagonal H sub-sell (with chain rotation) was followed. Notably, the two strong reflections characteristic of the $O_{\perp}$ sub-cell at 4.12Å (110) and 3.71Å (200) show progressive shifts in position and intensity, finally merging to the strong reflection at 4.20Å [(010) ≡ (100)] characteristic of the H sub-cell. Similar calculations are being performed for the other sub-cell types. Eventually we hope to use this approach to analyze the x-ray diffraction data of the HC packing modes characteristic of membrane phospholipids such as phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine.

W-Pos105 MEASURED HYDRATION ENERGY OF THE AQUEOUS CAVITIES OF THE PHOSPHOLIPID INVERTED HEXAGONAL PHASE. R. P. Rand, N. L. Fuller, Brock University, St Catharines, L2S 3A1 Canada and V. A. Parsegian, National Institutes of Health, Bethesda, MD 20205 USA

Hydration repulsion has been shown to dominate interactions between hydrophilic surfaces within separation of about 30 A. One measured property of interacting phospholipid bilayers is a variable coupling of the hydration energies between and within the lipid leaflets; i.e., upon dehydration, both interbilayer spacing and area/molecule decrease (Biophys. J. 37:657). It has been difficult to separate these two effects in order to determine for example the hydration energy of the isolated bilayer and its contribution to bilayer lateral pressure (Biophys. J. 37:667). Furthermore, for water cavities within inverted micelles, proteins or aqueous channels, where hydration energies are likely similarly important, geometry necessarily couples changes in molecular area and cavity dimension. We have measured the osmotic work required to dehydrate such a tightly coupled system by controlled osmotic stress of the inverted hexagonal phase formed by dioleylphosphatidylethanolamine (DOPE) in water. The cylindrical water cavities change diameter from 45 to 12 A and molecular area decreases from 54-33 A as log(applied stress, dynes/cm^2) increases to 8.5. Using in part the formalism of Kirk et al. (Biochemistry 23:1093), we are attempting to determine the contribution of (a) hydration, (b) interfacial elasticity, and (c) chain packing stresses to the measured work of deforming this lattice. To do this we are observing the effects on the lattice dimensions of added lipids that spontaneously assemble in aggregates of small curvature and of alkanes that relax chain packing stresses.

W-Pos106 The interaction of calcium with ganglioside G_{M1}. Robert V. McDaniel and Stuart McLaughlin. Physiology/Biophysics Dept., Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11794.

Other investigators have measured the binding of Ca to G_{M1} micelles in solutions of low ionic strength. Under these non-physiological conditions, the apparent association constant for the Ca-G_{M1} complex is high (e.g. 10^6 M^{-1}). However, most of the "bound" Ca could be sequestered in the electrical diffuse double layer adjacent to the negatively charged micelles rather than bound specifically to the G_{M1} head groups. We studied the binding of Ca to bilayer membranes containing 17 mole % G_{M1} in phosphatidylcholine (PC). We measured the electrophoretic mobility of PC:G_{M1} vesicles and the nonactin-K conductance of PC:G_{M1} planar bilayers in 0.1 M NaCl and KCl for $0<[Ca]<0.1$ M. We also substituted monoolein for PC or dimethonium for Ca. Ca binds to PC but not to monoolein. Dimethonium is a divalent cation that binds to neither PC nor monoolein. We described our results by combining the Poisson-Boltzmann and Navier-Stokes equations with the Langmuir adsorption isotherm. We conclude that Ca binds weakly to G_{M1} with an intrinsic 1:1 association constant of <100 M^{-1}, which is similar to the association constant of Ca with charged phospholipids. We obtained similar results with the gangliosides G_{D1a} and G_{T1b}.
Supported by NIH grant GM24971 and NSF grant PCM 8200991.

W-Pos107 PROPERTIES OF MIXED-CHAIN PHOSPHATIDYLCHOLINE ASSEMBLIES. Jeffery T. Mason, Frances A. Stephenson, and Ching-hsien Huang. Univ. of Virginia, Charlottesville, VA 22908

Differential scanning calorimetry and ^{31}P-nuclear magnetic resonance spectroscopy have been employed to investigate the physical properties of 1-steroyl-2-acetyl-sn-glycero-3-phosphorylcholine (C(18):C(2)PC) assemblies in excess water. Above 20°C, this highly asymmetric phospholipid forms a homogenous population of micelles with a hydrated radius of about 42 Å. When incubated between 0 and 14°C, the micelles slowly transform into an interdigitated gel lamellar phase. During this transformation, four distinct phases can be observed: micelles (I), aggregated micelles (II), a lamellar nucleation phase characterized by lamellar structures embedded in a matrix of micelle-like lipid (III), and finally the gel lamellar phase (IV). The kinetics of these transformations display a strong negative temperature dependence. Additionally, when the gel lamellar phase of C(18):C(2)PC is incubated at -18°C for 24 hr a crystalline lamellar phase (V) is observed. When the lamellar phase of this lipid is heated from 0 to 25°C three distinct endotherms are discernible. The lowest transition (T_m=8.1°C) is a solid-solid transition of the hydrocarbon chains of the lamellar assembly but may also involve changes in phosphorlycholine headgroup orientation and hydration. This transition is still present, but at a higher temperature (T_m=13.9°C) if the lipid is scanned from the crystalline phase rather than the gel phase. A second transition (T_m=17°C) has been tentatively identified as a gel lamellar $\leftrightarrow$ liquid crystalline lamellar transition. This transition is then closely followed (T_m=18.4°C) by the liquid crystalline lamellar $\rightarrow$ micellar transition. (Supported, in part, by Research Grant GM-17452, USPHS)

W-Pos108 PORPHYRIN MOBILITY ACROSS PHOSPHOCHOLINE PLANAR BILAYERS.
Martin C. Woodle and David Mauzerall, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Although there are many reports of charge movement across lipid bilayers doped with metalloporphyrins (MP), there has been no direct determination of the transit time of a charged species such as $MP^{+\cdot}$ across the bilayer. We have therefore examined a planar lecithin bilayer containing Mg-octaethylporphyrin which separates aqueous solutions of acceptor, ferricyanide or methyl viologen, and donor, ferrocyanide. A <1 μs pulse of light forms $MP^{+\cdot}$ and reduced aqueous acceptor, producing a photovoltage which decays in 10 μs to 100 ms, depending on the acceptor and the concentration of donor in the acceptor solution. When an electric field is applied across the bilayer, a photocurrent whose polarity follows that of the applied field rises in about 0.1 sec after the flash and decays with the membrane RC time constant. Since the addition of ferrocyanide to the side opposite to the acceptor fails to trap any $MP^{+\cdot}$ and the magnitude of the current correlates with the presence of oxygen, we attribute most of the current to superoxide anion and the remainder to impurities. With a pH gradient applied across the bilayer, we observed only 5% of the photovoltage expected if H^+ or OH^- are transported by $MP^{+\cdot}$. We conclude that $MP^{+\cdot}$ does not cross this bilayer system in <0.1 s.
This work was supported by PHS Grant GM 25693-06.

W-Pos109 MICROWAVES STIMULATE DRUG RELEASE FROM LIPOSOMES. Robert P. Liburdy, Lawrence Berkeley Laboratory, Division of Biology & Medicine, 1 Cyclotron Rd, Berkeley CA 94720 and Richard L. Magin, Dept. of Electrical Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Microwaves (2450 MHz, CW) are shown to stimulate rapid drug release from large unilamellar liposomes. This effect is unusual in that it occurs for exposures conducted below the lipid phase transition temperature where these liposomes are not leaky when heated by conventional means. The mechanism of interaction for this membrane response is not known; however, the effect is a linear function of absorbed power (3-60 mW/g) and is potentiated by blood plasma and oxygen. These results suggest a means for rapid and localized drug delivery in vivo. The phenomena may also provide a useful model for studying the interaction mechanisms operating between nonionizing electromagnetic radiation and biological membranes. Supported by ONR contract N00014-81K-0669 (RPL) and PHS Grant CA-29010 awarded by the NCI, DHHS (RLM).

W-Pos110 THERMOTROPIC PHASE BEHAVIOR OF LIPID BILAYERS COMPOSED OF PHOSPHATIDYLCHOLINES CONTAINING METHYL ISOBRANCHED FATTY ACIDS. R.N.A.H. Lewis, H.H. Mantsch and R.N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

The thermotropic phase behavior of aqueous dispersions of phosphatidylcholines (PC's) containing one of a series of methyl isobranched fatty acids was studied by DSC and ^{19}F- and ^{31}P-NMR and IR spectroscopy. The longer-chain PC's exhibit two endothermic phase changes, a slow, lower-temperature, lower-energy transition between two different gel states and a fast, higher-temperature, higher-energy chain-melting phase transition. These transitions converge and apparently become concomitant with decreases in hydrocarbon chain length. The longer-chain PC's form a partially dehydrated, highly ordered state at low temperatures, resembling the L_c phase of long-chain *n*-saturated PC's. At higher temperatures a fully hydrated, loosely packed gel state is formed, which is similar to but not identical with the $P_{\beta'}$-phase of their linear saturated analogues. The liquid-crystalline states of *n*-saturated and methyl isobranched PC's are generally similar but do show subtle differences in hydrocarbon chain organization. The gel to liquid-crystalline phase transition temperatures fall on a smooth curve but the gel/gel transition temperatures exhibit a pronounced odd/even alternation which increases in magnitude with increasing chain length. There are significant differences between the odd- and even-numbered isoacyl PC's with respect to hydrocarbon chain packing as well as headgroup mobility in their low-temperature gel states.
(Supported by grants from the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research)

W-Pos111 A THEORETICAL MODEL OF THE RIPPLE PHASE OF LIPID BILAYERS. Pao-Han Wang and L.E.H. Trainor. Dept. of Physics, University of Toronto. Toronto, Ontario, Canada M5S 1A7.

Some lipid molecules, such as DPPC possess a large polar headgroup and a hydrophobic tail consisting of two hydrocarbon chains. They form bilayers in excess water. Many experimental investigations, for example, using x-ray diffusion, differential scanning calorimetry and so on, have shown that at a certain temperature below the chain-melting transition temperature, a spatially periodic "ripple" phase appears. We put forward a statistical mechanical model to explain the ripple phase transition. Following Pearce and Scott (J. Chem. Phys. 77:951, 1982), we regard the bilayer as consisting of pairs of lipid molecules aligned vertically tail to tail - so-called "bimolecules" - situated on a square periodic NxN lattice. The state of a bimolecule at site (i,j) on the lattice in the xy plane may be described by means of a pair of variables (σ_{ij},μ_{ij}), in which $\sigma_{ij} = \pm 1$ describe the direction of the headgroup and $\mu_{ij} = \pm 1$ the relative vertical displacements of two adjacent "bimolecules". A very general model Hamiltonian is proposed. We consider short range interaction up to nearest neighbours in the j-direction and to next nearest neighbours in the i-direction. As a first step we use a mean field approximation to solve the model Hamiltonian, demonstrating the presence of the ripple phase in lipid bilayers. In addition, we examine the stability of the ripple phase and the configuration of the ground state of the system. Further study of this model is in progress, using renormalization group methods.

W-Pos112 LIPID BILAYER ASSEMBLY IN CELL MEMBRANES

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"Surface bilayers" (N.L. Gershfeld and K. Tajima, Nature, 279 708 (1979) form spontaneously in water with lipids that have been extracted from human erythrocytes, and from mesophilic and thermophilic bacteria that have been grown at different temperatures. The temperature of surface bilayer formation occurs at body temperature for the red blood cell lipids, and at the bacterial growth temperatures for each of the bacterial membrane lipid preparations. The thermodynamic properties of the surface bilayer system indicate that it is a critical state consisting of unilamellar lipid bilayers. The critical temperature for this system changes with lipid composition just as bacterial membrane lipids change with growth temperature. These properties of the surface bilayer system appear to satisfy essential requirements for lipid bilayer assembly in cell membranes.

W-Pos113 SIMULTANEOUS MODELLING OF PHASE AND CALORIMETRIC BEHAVIOUR IN AN AMPHIPHILIC PEPTIDE-PHOSPHOLIPID MODEL MEMBRANE M.R.Morrow, J.H.Davis, and J.C.Huschilt (Intro. by K.R. Jeffrey) Biophysics Interdepartmental Group, Physics Department, University of Guelph, Guelph, Ont. CANADA N1G 2W1

High sensitivity differential scanning calorimetry (D.S.C.) measurements have been performed on the amphiphilic peptide/DPPC-d62 systems for which partial phase diagrams have previously been determined by deuterium N.M.R. difference spectroscopy (1). Regular solution theory has been adapted to this system. It is shown that the peptide concentration dependence of the transition enthalpy can be incorporated into a model which reproduces the observed phase behaviour reasonably well without invoking the concept of "boundary lipid". The standard chemical potential of the dilute peptide is interpreted in terms of the perturbation of the bilayer by insertion of the peptide. The thermodynamic parameters determined by the simultaneous fitting of the phase behaviour and the transition enthalpies are used in a simulation of the D.S.C. scan shapes. While it is found that the asymmetry of the calorimetric scans for less than 2 mol % peptide is reproduced by the model, the broad component observed for higher concentration is not. It is postulated that this is an example of a situation in which a solute molecule decouples the first order melting transition from a continuous lattice expansion (2). (Supported by the Natural Sciences and Engineering Research Council, Canada. MM acknowledges the support of an Izaak Walton Killam Memorial Postdoctoral Fellowship.)

(1) J.C.Huschilt, R.S.Hodges, and J.H.Davis, accepted for publication in Biochemistry
(2) T.J.O'Leary B.B.A. 731 (1983) 47

W-Pos114 ADSORPTION OF Ca^{2+} TO SPHINGOMYELIN BILAYERS. W. Tamura-Lis, B.A. Cunningham, and L.J. Lis, Department of Physics and Liquid Crystal Institute, Kent State University, Kent, Ohio and J.M. Collins, Department of Physics, Marquette University, Milwaukee, Wisconsin.

The presence of divalent cations (Me^{2+}) in solutions of low molarity (1 to 100 mM) has been shown to cause phosphatidylcholine (PC) multibilayer arrays to swell to a greater extent than in water (Lis, et al (1981) Biochemistry 20:1771-1777). It was inferred that this behavior was caused by the adsorption of Me^{2+} to the PC head groups resulting in a charged bilayer surface. We have used x-ray diffraction to study the effects of Ca^{2+} in solution on bilayers made from the choline containing sphingolipid, sphingomyelin. The presence of 30mM $CaCl_2$ causes sphingomyelin bilayers to increase their bilayer separation from approximately 40Å observed in pure water to greater than 100Å. These results indicate that Ca^{+2} reacts in a similar manner with the structurally analogous head group moieties of the sphingosine based sphingolipids and the glycerol based phospholipids. In addition, we have used osmotic pressure and x-ray diffraction to deduce the relationship between applied pressure and bilayer separation for sphingomyelin bilayers in water and 30mM $CaCl_2$. Comparisons are made between these results and those previously reported for PC bilayers in water (Lis, et al (1982) Biophys. J. 37:657-672) or 30mM $CaCl_2$.

W-Pos115 THE INFLUENCE OF OXIDIZED STEROL COMPOUNDS ON DIPALMITOYLPHOSPHATIDYLCHOLINE STRUCTURE AND PACKING. M. Rooney and J.W. Kauffman, Biomedical Engineering Division, Northwestern University, Evanston, Illinois, W. Tamura-Lis and L.J. Lis, Department of Physics and the Liquid Crystal Institute, Kent State University, Kent, Ohio, S. Yachnin, Pritzker School of Medicine, University of Chicago, Chicago, Illinois, and O. Kucuk, The Chicago Medical School, North Chicago, Illinois.

An increase in the amount of oxidized sterol compounds (OSC) in cell membranes is observed in a number of disease states. X-ray diffraction and vibrational spectroscopies (FTIR and Raman) have been used to examine the interactions of a number of OSC on the bilayer structure and packing of dipalmitoylphosphatidylcholine bilayers. Sterols which have been oxidized in the 7-position produce a condensing effect on the DPPC bilayer, as shown by FTIR and Raman Spectroscopies, which is intermediate to the effect observed on DPPC bilayers with cholesterol and without cholesterol. The infrared fingerprint region revealed that head group mobility in the 7-ketocholestanol/DPPC bilayers is significantly reduced compared to the other sterol/DPPC systems. The vibrational data also indicate that the effects of the 7-position sterols on DPPC can be amplified in the presence of cholesterol suggesting unique sterol/cholesterol stoichiometries in bilayer packing subcells. All other oxidized sterols studied have approximately the same effect as cholesterol on DPPC bilayer packing. X-ray diffraction studies show that DPPC bilayers containing sterols oxidized at the 7-position have different swelling properties than pure DPPC and DPPC-cholesterol bilayers. For example, DPPC/7-ketocholesterol (9:1) mixtures produce one lamellar phase with a repeat spacing of approximately 80 Å at full hydration in water, while DPPC/cholesterol (9:1) mixtures produce two lamellar phases with repeat spacings at full hydration of approximately 64 Å and 80 Å.

W-Pos116 INFLUENCE OF CATION SPECIES ON THE FORCES BETWEEN PHOSPHATIDYLCHOLINE BILAYERS. B.A. Cunningham, W.M. Kwok, and L.J. Lis, Department of Physics and the Liquid Crystal Institute, Kent State University, Kent, Ohio 44242.

The influence of cation species in salt solutions on the hydration and van der Waals forces acting between dipalmitoylphosphatidylcholine surfaces using multi-lamellar arrays was examined. Osmotic pressure was used to obtain the net repulsive pressure between bilayer surfaces, and x-ray diffraction was used to determine bilayer structural parameters. Initial studies (S. Afzal, et al (1981) J. Coll. Interface Sci 97:303) showed that increasing the KCl or NaCl concentration in the aqueous compartment between DPPC bilayers lowered both the hydration and van der Waals forces. The influence of one molar solutions of chloride salts (Na^+, K^+, Li^+, Cs^+, NH_4^+ and Ba^{+2}) or potassium salts (Cl^-, $SO_4^=$ and $Acetate^-$) on the forces between DPPC bilayers was then examined. Preliminary studies indicate that Li^+, alone, appears to have no effect on the interactive forces when compared with the values obtained for DPPC bilayers in water. In all cases, ion species or concentration had no measurable effect on the bilayer thickness at full hydration, or on the bilayer thermodynamic properties. These results are consistent with ion-produced pertubations of the solvation layers between phospholipid bilayers.

W-Pos117 THERMAL AND PERMEABILITY PROPERTIES OF DILAURYL PHOSPHATIDYLETHANOLAMINE (DLPE) DISPERSIONS. S. Melnick[a], L. Finegold[b], and M. Singer[a] a Dept. of Medicine, Queen's Univ., Kingston, Ont. K7L 3N6 b Dept. of Physics, Drexel Univ., Philadelphia, PA 19104.

The thermal and permeability properties of DLPE dispersions were compared with regard to such factors as method of liposome preparation and commercial source of lipids. Multilamellar liposomes were prepared by hydrating lipid at either 50°C or 80°C using DLPE from the Sigma Chem. Co. and Calbiochem. Corp. Samples were judged comparable by thin lay chromatography, gas chromatography, and infrared spectroscopy. Na^{22} permeability was studied between 20°C and 55°C immediately following liposome preparation and also after three months storage at 5°C. Differential scanning calorimetry (DSC) was performed using a Mettler TA 2000B modular system. Freshly prepared liposomes displayed an endotherm at 30°C independent of lipid source and hydration temperature but the permeability versus temperature profiles were sensitive to both of these factors. Liposomes stored in the cold for 1 - 3 months manifested gel and liquid crystal metastability with new endotherms appearing at 35°C and 44°C, depending on hydration history and lipid source. However the permeability properties of stored liposomes from both sources tended toward a similar profile with efflux maxima at 30°, 39°, and 44°C. This study demonstrates that the thermal and permeability properties of DLPE dispersions are sensitive to the lipid and liposome preparation history and that DSC and efflux measurements give complementary information with regard to membrane properties.

W-Pos118 THE EFFECT OF URANYL NITRATE ON THE ELECTROKINETIC PROPERTIES OF MODEL MEMBRANES AND HUMAN ERYTHROCYTES. Louis Pasquale & Stuart McLaughlin. Dept. of Physiology & Biophysics, HSC, SUNY Stony Brook, NY 11794.

We studied the effect of uranyl nitrate on the electrophoretic mobility of (i) egg phosphatidylcholine (PC) vesicles, (ii) vesicles formed from a mixture of PC and either phosphatidylserine (PS) or the ganglioside G_{M1}, and (iii) human erythrocytes. Most of our measurements were made in 0.15 M NaCl at pH 5. We used the Helmholtz-Smoluchowski equation to express the mobility in potential units. 0.1 mM uranyl nitrate increases the zeta potential of PC vesicles from 0 to +40 mV. This positive zeta potential is due to the specific adsorption of UO_2^{++} to the phosphate group of PC with an intrinsic association constant of about 10^6 M^{-1}, a result that agrees with the association constant measured by other investigators using different techniques. UO_2^{++} does not change the electrophoretic mobility of vesicles formed from mixtures of the neutral lipid monolein and the ganglioside G_{M1}, which demonstrates that it does not adsorb to the sialic acid residues. Uranyl nitrate has similar effects on the electrophoretic mobility of human erythrocytes and PC:G_{M1} vesicles: 0.1 mM UO_2^{++} changes the zeta potential of the erythrocytes from -13 to +8 mV and that of PC vesicles containing 20 mole % G_{M1} from -11 to +5 mV. In contrast, 0.1 mM uranyl nitrate has a much larger effect on the zeta potential of PC vesicles containing 9 mole % PS: the zeta potential increases from -13 to +37 mV. These results demonstrate that membranes containing the ganglioside G_{M1} can be used to mimic the electrical properties of biological membranes. Supported by NSF grant PCM-8200991 and NIH grant GM24971.

W-Pos119 RAMAN SPECTROSCOPIC STUDY OF THE CALCIUM INDUCED PHASE SEPARATION IN PHOSPHATIDYLCHOLINE - PHOSPHATIDIC ACID MIXTURES. M. Pézolet, R. Kouaouci, J. Silvius*, I. Graham+, and M. Zuckermann+. Département de chimie, Université Laval, Québec; * Department of Biochemistry and + Department of Physics, Montréal, Québec, Canada.

Raman spectroscopy has been used to investigate the effect of calcium ions on mixtures of the zwitterionic phospholipid DMPC and the anionic lipid DMPA. By using one component of the mixtures with fully deuterated acyl chains, it was possible to determine simultaneously the conformation of each lipid by looking out either the C-H or C-D stretching regions. The C-H stretching region was monitored by either the h_{2880}/h_{2850} or the h_{2930}/h_{2880} intensity ratios while in the C-D stretching region, the linewidth of the 2103 cm^{-1} band was used. DMPA and DMPC-d_{54} are highly miscible and form essentially an ideal solution at a 1:1 molar ratio since the gel to liquid-crystalline phase transition of this mixture appears at the average temperature of those of the pure lipids. Equimolar mixtures of DMPC and DMPA-d_{54} exhibit the same behavior. In the presence of excess of calcium ions, the temperature profiles obtained from the two spectral regions are quite different. For example, the C-H region for an equimolar mixture of DMPA and DMPC-d_{54} shows that DMPA is in a tightly packed lattice and does not give rise anymore to a phase transition. These features are characteristic of the dehydrated phase of anionic phospholipids in the presence of divalent cations. Therefore, most of the DMPA component of the mixture is complexed with calcium. On the other hand, the melting curve obtained from the C-D region in the spectrum of DMPC-d_{54} demonstrates that this lipid is phase separated from DMPA since it gives a transition that is at essentially the same temperature as that of pure DMPC-d_{54}. These results will be compared with those obtained by high sensitivity calorimetry.

W-Pos120 The Effects of pH and Divalent Cations on the Phase Behavior of Liposomes Composed of Phosphatidylethanolamine and Fatty Acid. Jerome Connor, Leaf Huang, Dept. of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

Dioleoyl phosphatidylethanolamine (DOPE) and oleic acid (OA) in an 8:2 molar ratio will form stable sonicated vesicles at neutral pH. The transition of these liposomes from a bilayer structure to a hexagonal II configuration was monitored by ^{31}P NMR. Decreasing the pH below neutral caused a lipid phase transition into the hexagonal phase. Increasing proton concentrations increased proportionally the amount of lipid which displays a hexagonal conformation; at pH=6 the lipid exists totally in the hexagonal phase. The addition of 2 mM Ca^{++} to liposomes at neutral pH causes approximately 40% of the lipid to undergo the transition to hexagonal phase; increasing the Ca^{++} concentration to 4 mM induces about a 60% hexagonal phase composition. Mg^{++} is less effective; 6 mM Mg^{++} cause only 15% of the lipid to display the hexagonal phase. Ca^{++} also acts synergistcally with protons in initiating the hexagonal phase. The additions of Ca^{++} increases the pH at which the bilayer to hexagonal phase transition is seen. This synergistic effect of Ca^{++} and protons can also be seen by monitoring lipid transfer using a resonance energy transfer assay. The existence of the hexagonal phase of this lipid composition at low pH has also been demonstrated by freeze-fracture electron microscopy. (Supported by NIH Grant CA 24553).

W-Pos121 TEMPERATURE DEPENDENT PERMEABILITY OF LIPOSOMES UNDER MICROWAVE IRRADIATION Michel OLLIVON and *Patrick VERDIER. O.M.M.-GR 35, C.N.R.S., BP 28, 94320 Thiais, France, *I.T.S.-Universite de Compiegne, BP 233,60206 Compiegne, France. Introduced by Dr. Anne Walter

The influence of high frequency electric field on the bilayers has been studied by fluorescence, measuring the permeability of large unilamellar vesicles using calcein-cobalt system. Encapsulated volumes of 15 to 20 l/mole, found by addition of cobalt chloride to calcein containing liposome solution, agree with iodinated tyrosine measurements, and with the size distribution (300-500 nm) measured by electron microscopy. High sensitivity differential calorimetry has been used to characterize the main phase transition of the liposomes made from mixtures of DPPC and DSPC. Microwave irradiation (7W at 2.43 Ghz) and fluorimetric measurements were simultaneously performed in the same sample using a resonant cavity. Under these conditions the electric field in the sample as well as the dielectric losses are maximized. A fluorescent thermometer has been used to avoid any perturbation of the electric field. With liposomes made from DPPC/DSPC (80/20) mixtures no detectable changes of the encapsulated volumes was measured after 8 months at 4°C. Furthermore, at 10°C no detectable permeability has been measured. Near the pretransition temperature the permeability increased gradually with temperature but increased rapidly near the main transition. At this point the permeability seemed to be maximum during heating but minimum at stationary temperature. Permeability changes from microwave heating secondary to dielectric losses, were found to be identical to conventional heating effects to within 1°C. Therefore, we deduce that neither the high frequency electric fields nor associated polarization resulting from microwave irradiation cause appreciable leakage from vesicles.

W-Pos122 GATING OF A VOLTAGE-DEPENDENT CHANNEL (COLICIN E1): THE ROLE OF MEMBRANE TRANSLOCATION OF PROTEIN. Lynn Raymond, Stephen L. Slatin, Alan Finkelstein. Albert Einstein College of Medicine, Bronx, N.Y.

Colicin E1, a water soluble protein, forms voltage-dependent, ion-selective channels in planar lipid bilayers. Since the gene for colicin E1 has been cloned and sequenced, making the protein a target for both genetic and chemical modification, E1 is an ideal model for studying structure-function relationships of channel gating. Various channel-forming, C-terminal fragments of colicin E1 can be generated by standard methods (Cleveland et al, 1983); the shortest of these fragments (produced by cyanogen bromide cleavage) is 152 amino acids (MW ca. 16 KD). A somewhat larger (ca. 18 KD) C-terminal fragment (ThCF) is generated by the protease thermolysin. These three channels (E1, ThCF, CNBr) can be distinguished by differences in kinetics at pH's above 5, where CNBr gates faster than ThCF which gates faster than E1. Treatment with trypsin on the membrane converts gating behavior of longer peptides to that characteristic of shorter fragments. Apparently trypsin removes segments of protein N-terminal to the channel-forming region, since gating behavior of the cyanogen bromide fragment is little affected by the enzyme. More striking, however, is that this conversion from longer to shorter peptides occurs when trypsin is added cis (the side to which protein has been added) only when channels are in the closed state, whereas channels must be open for conversion from the trans side. These results suggest that tryptic cleavage sites are being transferred across the bilayer as channels open and close. Evidently, channel gating involves translocation of large protein segments which are themselves not necessary for channel formation but do affect on/off rates. (Supported by NIH grant GM29210 and T327288 from NIGMS).

W-Pos123 INACTIVATION OF A VOLTAGE-DEPENDENT CHANNEL (COLICIN E1): THE ROLE OF MEMBRANE TRANSLOCATION OF PROTEIN. S.L. Slatin, L. Raymond, A. Finkelstein. Albert Einstein Coll. of Med., Bronx, N.Y. The E1 group of colicins are bacterial proteins that form voltage-dependent ion channels in membranes. Colicin E1 opens in response to cis [the side to which colicin is added] positive voltage and closes in response to cis negative voltage. However, at very low pH (3.5) and large positive potentials (>80 mV), channels that are already on turn off into a different off state, i.e., they inactivate. Under some conditions inactivated channels never reappear, but under other conditions they can open again, albeit at a much slower rate than normal off channels. Interestingly, some of the reactivated channels seem to reappear on the trans side. These "flipped" channels behave like normal channels except that their voltage dependence has been reversed. The following experiment addresses the issue of whether this reversed voltage dependence is due to the protein being driven across the membrane, or to a more subtle rearrangement of the gating region. At low pH, the endopeptidase pepsin added to the cis solution destroys channels, but only when they are off. Trans pepsin has no effect on channels that are either on or in the normal off state. This implies 1) normal off/on gating works by exposing and "unexposing" part of the protein to the cis solution (perhaps by inserting protein into the membrane), and, 2) pepsin is a test for "cisness" of normal E1 channels. Trans pepsin was found to destroy "flipped" channels, implying that these channels see the pepsin as cis. Also, flipped channels are protected from cis pepsin. These results suggest that flipped channels are normal channels that have crossed the membrane. In the inactivated state the protein may be intermediate between the on state, which must span the bilayer, and the flipped state. (NIH GM29210 and T327288 from NIGMS).

W-Pos124 MOLECULAR MODELS AND IN VITRO MUTAGENESIS OF COLICIN E1. Q.-R. Liu, R. Fine, V. Crozel, F. Levinthal and C. Levinthal. Columbia University, New York, NY 10027.

Colicin E1 is a protein produced by certain strains at E. coli which kills sensitive cells by discharging their membrane potential.

Molecular model building and oligonucleotide mutagenesis have been used to study the structure-function relationships of that portion of the colicin E1 protein which makes ion channels in phospholipid membranes. By introducing methionine codons to provide a site for CNBr cleavage we can vary the location of the amino-terminus of the channel peptide fragment. Similarly, by introducing stop codons the position of the carboxy-terminus can be varied. To date, the shortest active fragment has 129 amino acids. This size seems inconsistent with a model of the structure which is a barrel of six alpha helices each of which would have to be about 20 amino acids long in order to span the membrane. Additional mutations have been made which introduce changes in the number and location of charged residues within the active peptide. These also suggest that the structure is not made of alpha-helices.

We are currently testing the hypothesis that the channel is composed of a single layer β sheet with ten strands formed into a twisted barrel. The tests involve producing mutants each of which has a cys residue at different positions along the polypeptide chain so that sulfhydryl reagents can be used to determine whether particular residues face into the channel lumen or into the lipid region of the membranes.

W-Pos125 PHOTOLYSIS OF GRAMICIDIN IN METHANOL, VESICLES, AND PLANAR BILAYERS. Daniel Jones, Alan Miller, Syed Husain, David Busath and Elie Hayon. Sect. of Physiol. and Biophys., Brown University, Providence, RI 02912 and Dept. of Chemistry, Queens College, Flushing, NY 11367.

The fluorescence emission spectrum of gramicidin D dissolved in methanol (0.01 mg/ml) was compared to that of gramicidin D solubilized in DMPC vesicles (0.01 mg peptide, 0.1 mg lipid/ml water). Using 280 nm excitation, the peak fluorescence occurred at 358 nm in methanol as expected for peptide tryptophans, and at 349 nm in vesicles suggesting a less polar Trp environment. The peak amplitude in vesicles was about 60% of that in methanol. The vesicle spectrum contained an extra peak at 448 nm which was present in vesicles without peptide but not if the lipid was dissolved in methanol. We compared the effect of broad-spectrum ultraviolet illumination of the two samples to examine the role of the peptide environment in the photolysis rate measured by fluorescence. The Trp fluorescence peak declined exponentially upon UV exposure in both cases without effect on the peak shape or location. Time constants were 21 seconds in methanol and 19 secs in vesicles. The vesicles also showed a minor fast component. The peak at 448 nm was unaffected by the UV exposure. For comparison we measured the conductance decay under similar illumination conditions with planar lipid bilayers (GMO/hexadecane) containing many gramicidin channels. Following a small early component, the conductance during UV exposure declined exponentially with a time constant of 12 seconds. Aside from the small fast component in bilayers, these results suggest that gramicidin tryptophan photooxidation proceeds at about the same rate in methanol as in vesicles, ie., is independent of peptide conformation and environment. The higher rate of conductance photolysis is consistent with the multiple tryptophans per channel.

W-Pos126 ENERGY TRANSFER BETWEEN GRAMICIDIN AND ANTHROYLOXY FATTY ACIDS. A.J. Connolly, L.T. Boni and A.M. Kleinfeld, Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.

Resonance energy transfer was used to investigate the membrane disposition of gramicidin. Gramicidin D was reconstituted into small unilamellar vesicles of dimyristoylphosphatidylcholine at lipid to peptide mole ratios between 30:1 and 180:1 and at all times vesicles were maintained at temperatures above 30°C. The n-(9-anthroyloxy) (AO) fatty acids (n between 2 and 16) were added to the vesicles in probe-lipid mole ratios between 0.5 and 5%, and energy transfer efficiencies were determined between the peptide's tryptophan (Trp) and the AO probes. Good agreement was obtained between transfer efficiencies determined from Trp quenching and those from sensitized emission of the AO probes. Lifetime and steady state measurements indicate that direct AO fluorescence is unaffected by the presence of gramicidin. Steady state polarization values vary between 0.09 (290 nm) and 0.20 (305 nm), indicating that at least some of the Trp are partially mobile. Since the necessary conditions for application of the Forster theory are satisfied, a Monte Carlo adaptation of the theory was used to analyze the transfer between the tryptophans of gramicidin and each of AO probes. Preliminary analysis, assuming a quantum yield of 0.11 (the peptide average) for all Trp, is consistent with an N to N configuration of the peptide dimer. Measurements are currently in progress to estimate the heterogeneity of the Trp quantum yields using n-bromosuccinimide quenching and oxidation of tryptophanyl residues. This work was supported by NSF grant PCM-8302687 and was done during the tenure of an Established Investigatorship of the American Heart Association.

W-Pos127 GRAMICIDIN K: A NEW LINEAR CHANNEL-FORMING GRAMICIDIN FROM BACILLUS BREVIS. William L. Whaley, Jean A. Paczkowski and Roger E. Koeppe II, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701; and Julie Weiss and Olaf S. Andersen, Department of Physiology and Biophysics, Cornell University Medical College, New York, NY 10021.

A new linear gramicidin has been isolated from a commercial mixture of gramicidins A, B and C. This new molecule, designated gramicidin K, has a molecular weight 20% higher than gramicidin A, contains formyl and ethanolamine functional groups, and is strongly retained on reversed phase liquid chromatographic columns. Gramicidin K has the same amino acid composition as a mixture of gramicidins A and C: Gly, L-Ala, L-Val, D-Val, D-Leu, L-Trp, L-Tyr, and L-Ile in ratios of 1:2:2:2:4:3.7:0.3:0.1. Gramicidin K can be further resolved into two components, one of which contains one equivalent of tyrosine. ^{13}C and ^{1}H NMR spectra suggest the presence of a long aliphatic chain and a new ester bond in gramicidin K. Because the formyl and indolyl resonances are identical in gramicidins K and A, it is likely that the aliphatic chain is attached by means of an ester linkage to the ethanolamine group of gramicidin K. Both subtypes of gramicidin K form unique and long-lived cation-selective channels in lipid bilayer membranes. The small-signal (25-50 mV) single-channel conductances of the K-(Tyr) and K-(Trp) components are 9.9 and 12.1 pS compared to 12.1 and 12.4 pS for gramicidins C and A, respectively (1.0 M NaCl, diphytanoyl-PC). The mean channel lifetimes are 3-5 times longer than gramicidin A. Gramicidin K should prove to be useful for investigations of the biosynthesis of linear gramicidins as well as the channel activities of modified gramicidins.

W-Pos128 BROWNIAN DYNAMIC SIMULATIONS OF ION MOVEMENT IN A GRAMICIDIN-LIKE CHANNEL. Peter Gates and Eric Jakobsson, Department of Physiology and Biophysics, and Program in Bioengineering, 524 Burrill Hall, 407 S. Goodwin, University of Illinois, Urbana, IL 61801.

Previous work from our lab (1) has described one-dimensional movement of individual ions in membrane channels as Brownian motion, and demonstrated that this approach provides the detailed description to which both bulk electrodiffusion theory and Eyring rate theory (barrier-hopping models) are approximations. There is much evidence that in gramicidin channels ions do not move as individual entities but rather as part of a "plug" consisting of ions and water molecules. In the present work we show the extension of the Brownian dynamics method to the "plug" boundary condition and how it can be used to compute such features of gramicidin channels as streaming potentials, coupled water-ion movement, and the different apparent diffusion coefficients of water as measured by tracer studies and by movement down an osmotic gradient. We also will discuss the next step, which would be coupling the Brownian dynamics simulations to the molecular dynamic calculation of instantaneous (i.e., picoseconds relaxation time) forces exerted on ions in the gramicidin channel. (2) We believe this combination may be a promising route to a truly molecular description of events underlying measured fluxes in the gramicidin channel.

1) Cooper, K., Jakobsson, E., Wolynes, P. 1984. Prog. in Biophys. and Mol. Biol, in press.

2) Mackay, D. H. J., Berens, P. H., Wilson, K. R. and Hagler, A. T. 1984. Biophys. J. 46: 229-248.

Suport from the Research Board and the Bioengineering Program at the University of Illinois. P. G. is an NIH Cell and Molecular Biology Trainee.

W-Pos129 **ESTIMATION OF THE SODIUM PERMEABILITY OF GRAMICIDIN CHANNELS FROM ^{23}Na NMR STUDIES.** **N.R. Shochet*, C.S. Springer Jr.*, C. Clausen, L.C. Moore. Departments of Chemistry(*) and Physiology and Biophysics, State University of New York at Stony Brook.**

A method for estimation of ionic permeabilities of membranes from NMR ionic flux measurements was developed using a mathematical model of membrane ion transport. ^{23}Na NMR was used to monitor the sodium flow through gramicidin (G) channels in liposomes. Experiments were performed on phosphatidyl choline (PC) vesicles loaded with a 100 mM NaCl buffer and suspended in a Na-free buffer with varying amounts of KCl. The separation of the intra- and extravesicular Na NMR peaks was achieved by addition of a membrane impermeable shift reagent. Gramicidin was added to yield a G/PC molar ratio of $2x10^{-5}$. The time course of the G-induced efflux was obtained from changes in the relative peak areas in the NMR spectra which were obtained at 79.4 MHz by accumulating 128 FIDs with an acquisition time of 256 msec each. A dynamic model of vesicle ion transport was incorporated into a parameter estimation algorithm to extract the sodium permeability of the ionophore-treated vesicular membrane from the Na efflux data. The model assumes: a) only cations are permeable b) ionic fluxes can be described by the GHK equation c) the ratio of the Na/K permeabilities is 0.3 d) activity coefficients are 1.0. The membrane sodium permeability and the initial amount of intravesicular sodium were estimated. The model predicts the initial intravesicular sodium concentration to be 77.9 mM. The resulting mean sodium permeability estimate is $3.9x10^{-10}$ cm/sec and it increases with increased G/PC ratio. The calculated initial membrane potentials are 17.24 mV and 3.28 mV for 100 mM and 50 mM KCl in the external bath, respectively.

W-Pos130 DIFFERENTIAL BLOCK OF TWO TYPES OF CALCIUM CHANNELS IN NEUROBLASTOMA CELLS. Akinobu Tsunoo, Mitsunobu Yoshii and Toshio Narahashi (Intr. by S. F. Holloway). Dept. of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

Our recent study has shown that neuroblastoma cells (N1E-115) have two types of voltage-gated Ca channels: one generates a transient Ca current (type I) and the other generates a long-lasting Ca current (type II) (Soc. Neurosci. Abstr. 10, 527, 1984). We have found differential blocking actions on the two types of Ca channels of various polyvalent cations and the pyrethroid tetramethrin, a potent Na channel modulator. Ca channel currents as carried by Ba^{2+} (50 mM) were recorded using the whole-cell variation of the patch clamp technique. La^{3+}, Cd^{2+}, Ni^{2+} and Co^{2+} blocked both types of Ca channels in a dose-dependent manner with one-to-one stoichiometry. For the type I channel, the sequence of blocking potency as estimated from the apparent K_d (μM) was $La^{3+}(1.5) > Ni^{2+}(47) > Cd^{2+} \cong Co^{2+}(160)$. For the type II channel, the sequence was $La^{3+}(0.9) > Cd^{2+}(7.0) > Ni^{2+}(280) > Co^{2+}(560)$. Tetramethrin (50 μM) greatly reduced the amplitude of peak type I current by 70-80% while it only reduced type II current by 20-30%. In addition, it caused a time-dependent exponential block of both type I and type II channels. The time constants of this block seemed to be voltage independent, being approximately 75 msec for type I and 300 msec for type II channels. Verapamil (100-500 μM) caused a similar time-dependent block of type II channels, but had no time-dependent blocking effect on type I channels. The results provide further evidence that two different types of Ca channels coexist in the neuroblastoma cell. Supported by NIH grants NS14143, NS14144, RR05370 (M.Y.) and Muscular Dystrophy Association Fellowship (A.T.).

W-Pos131 DIFFERENT PROPERTIES IN TWO TYPES OF CALCIUM CHANNELS IN NEUROBLASTOMA CELLS. Mitsunobu Yoshii, Akinobu Tsunoo and Toshio Narahashi (Intr. by C. H. Wu). Dept. of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

We have previously shown that cultured neuroblastoma cells (N1E-115) have two types of voltage-sensitive Ca channels: one shows fast inactivation (type I) whereas the other shows only very slow inactivation (type II) (Soc. Neurosci. Abstr. 10, 527, 1984). We now report further differences in the gating and permeation properties of these two types of Ca channels. Ca channel currents as carried by Ba^{2+}(50 mM) were recorded using the whole-cell variation of the patch clamp technique. Activation of type I channels followed second order kinetics, whereas that of type II channels followed first order kinetics. The activation time constants of both types were voltage dependent, exhibiting similar bell-shaped curves. However, the peak occurred at different potentials; at -55 mV for type I and at -5 mV for type II (apparent activation levels: -55 mV for type I and -20 mV for type II). The steady-state inactivation curves were almost identical in shape (slope factor: 4.0-4.5 mV/e-fold change), but had different mid-points; -55 mV for type I and -20 mV for type II. The two types of channels also differed in their ionic selectivity as estimated from the peak current amplitude; Ba^{2+}: Sr^{2+}: Ca^{2+}= 1.0 : 1.0 : 0.6 for type I, and Ba^{2+}: Sr^{2+}: Ca^{2+}= 1.0 : 0.7 : 0.3 for type II. Replacement of Ba^{2+} with Ca^{2+} caused a positive voltage shift in the I-V relationship for type II, but not for type I. The Q_{10} values of the peak current amplitude were almost identical for types I and II. These results strongly suggest that the two types of Ca channels found in neuroblastoma cells represent different entities. Supported by NIH grants NS14144, RR05370 (M.Y.) and Muscular Dystrophy Association Fellowship (A.T.).

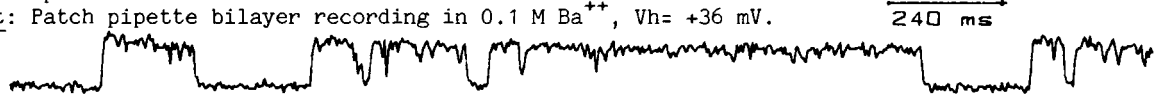
W-Pos132 FAST-KINETIC CHARACTERIZATION OF VOLTAGE DEPENDENT Ca^{2+} ENTRY IN SYNAPTOSOMES. T. Wang, M. M. Murawsky, and J. B. Suszkiw, Depts of Physiology and Biophysics and Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0575

Mammalian brain synaptosomes provide a useful in vitro system for investigation of biochemical regulation of presynaptic Ca^{2+} fluxes and Ca^{2+}-dependent transmitter release. Previous measurements on synaptosomes in the time range of ≥1sec indicated a biphasic K^+-activated Ca^{2+} entry and transmitter release, consisting of an initial, fast and a late, slow components (Nachshen and Blaustein, 1982, J. Gen. Physiol.; Suszkiw and O'Leary, 1983, J. Neurochem.). To further resolve and characterize the fast component of Ca^{2+} entry we have employed in the present study a rapid quench-flow/rapid filtration procedure which enabled us to extend the measurement of the time course of Ca^{2+} influx down to 50 msec of K^+ depolarization. Analysis of the initial rates of Ca^{2+} influx in synaptosomes depolarized with 52.5 mM K^+ at external calcium concentrations from 0.1 to 10 mM yielded a J_{Ca}^{max} = 6.1 ± 0.5 nmol/sec/mg protein and K_{Ca} = 1.4 ± 0.4 mM for the fast component, which inactivated with a half-time of 1 - 1.5 sec. Replacement of NaCl in the medium with choline Cl was without effect on the fast phase, but reduced the slow phase of K^+-stimulated Ca^{2+} entry. It is concluded from these results that (1) synaptosomes contain a single class of voltage-dependent Ca^{2+} channels which are activated by K^+-depolarization, remain open for 1 to 2 sec, and are half-saturated by Ca^{2+} at approximately the physiological $(Ca^{2+})_o$ and (2) Ca^{2+} accumulation in synaptosomes during longer depolarizations appears to be mediated by a membrane-potential sensitive Na^+-Ca^{2+} exchange mechanism, which becomes operative in synaptosomes artificially enriched in Na^+ prior to K^+ depolarization. (Supported by NIH Grant NS 20786)

W-Pos133 PLANAR BILAYER RECORDING OF SINGLE CALCIUM CHANNELS FROM PURIFIED MUSCLE TRANSVERSE TUBULES. Hubert Affolter and Roberto Coronado. Department of Pharmacology, University of North Carolina At Chapel Hill, Chapel Hill, NC 27514.

Bilayers formed in patch pipettes and in large apertures (BLM) were used to record single divalent-cation channels from rat skeletal muscle transverse tubule vesicles (membrane preparation of Rosemblatt et al, 1981). In patch pipettes filled with 0.1 M $BaCl_2$ and bath solution containing 0.2 M NaCl or CsCl, single channel currents have an amplitude of 0.7 pA at a holding of 0 mV. From single channel reversal data and GHK equation: P(Ba)/P(Na)=15.5, P(Ba)/P(Cs)=24.5, and P(Ba)≈P(Sr) ≈P(Ca). In PE:PS (1:1) bilayers (BLM data), single channel conductance saturates at 20 pS when cis Na^+>0.2 M or at 10 pS when cis Ba^{++}>0.05 M (cis is intracellular-equivalent side). Distributions of open and closed times are biexponential. From a sample of 3000 events at 0 mV: τ(short)=10 ms (closed events,c_s); τ(short)=12 ms (open events,o_s); τ(long)=900 ms (closed events,c_ℓ); τ(long)= 110 ms (open events,o_ℓ). Conditional distributions of events in time show that c_s-o_s transitions are correlated and lead to fast flickering. Channels escape from flickering via $o_s \rightarrow o_\ell$ or $c_s \rightarrow c_\ell$ transitions. Probability of channel openning is voltage-dependent increasing at cis positive holdings, although it seldom reaches unity. Supported by NIH GM-32824 and fellowship from Swiss National Funds.

Insert: Patch pipette bilayer recording in 0.1 M Ba^{++}, Vh= +36 mV.



W-Pos134 KINETICS OF DIHYDROPYRIDINE-SENSITIVE SINGLE CALCIUM CHANNELS FROM PURIFIED MUSCLE TRANSVERSE TUBULES. Roberto Coronado and Hubert Affolter, Department of Pharmacology, University of North Carolina At Chapel Hill, Chapel Hill, NC 27514.

Blockade by the calcium channel antagonists nitrendipine and D600, and activation by the agonist Bay K8644 were studied in divalent cation selective channels derived from rat skeletal muscle transverse tubules (membrane preparation of Rosemblatt et al,1981). Channels are recorded in PE:PS(1:1) planar lipid bilayers of the Mueller-Rudin type, using 0.25 M Na^+ (sodium medium) or 0.1 M Ba^{++}(barium medium) as current carrier (cis corresponds to the intracellular-equivalent side) Trans-ground solution was 0.05 M Na^+, 0.1 mM EGTA (or EDTA). In control records in sodium medium at 0 mV, bursts of open events averaged 120 ms; interburst period, 490 ms; and the mean open time, 50 ms. Cis addition of 1μM nitrendipine (Ki≈1μM): i) decreased the burst duration to 45 ms; ii) increased the interburst period to 1.5 sec; and iii) decreased the mean open time to <20 ms. Trans effects were also observed, however, they required markedly long drug equilibration times (>45 sec). Cis block by D600 (Ki≈20μM) was qualitatively similar to cis block by nitrendipine. Channels incorporated in the presence of cis 5 μM Bay K8644, either in sodium or barium media, have open lifetimes that are 2-5 times longer than in control records. Bay K channels have a similar voltage-dependence than those recorded in the absence of agonist; the open channel probability increases with cis-positive potentials. Amplitudes of open channel currents and reversal potentials are also the same. From a sample of 2560 events in Bay K at 0 mV using barium medium, distributions of open and closed lifetimes are biexponential: τo_s=25 ms; τo_ℓ=180 ms; τc_s=10 ms; and τc_ℓ=250 ms. Supported by NIH GM-32824 and fellowship from Swiss National Funds.

W-Pos135 ACTIVATION OF AN INWARD CURRENT IN DORSAL ROOT GANGLION CELLS BY TRANSIENT REDUCTIONS IN EXTRACELLULAR CALCIUM. John J. Hablitz, U. Heinemann and H.D. Lux (Intr. by R.J. Bick). Max-Planck Institute for Psychiatry, Planegg-Martinsried, FRG and Baylor College of Medicine, Houston, TX.

Removal of external calcium or the addition of calcium chelating agents induces a voltage-dependent slow inward current in molluscan neurons (Kostyuk et al., J. Memb. Biol. 76:83-93, 1983). We have examined whether transient changes in extracellular calcium alters whole cell currents and single channel currents recorded from cultured chick dorsal root ganglion cells.

Calcium-deficient solutions evoked an inward current in all neurons tested. The amplitude of this current was voltage dependent, decreasing as the holding potential was made less negative. No reversal was demonstrable over the range of voltages tested (-100 to +40 mV) when cesium was the major intracellular cation. A clear reversal near 0 mV was observed when intracellular sodium was raised. These induced currents were TTX insensitive. They were not observed in the absence of sodium or in the presence of cadmium. Exposure of outside-out membrane patches to calcium-deficient solutions induced openings of single, inwardly directed channels. Over the voltage range -100 to -20 mV, the slope conductance was approximately 10 pS. Both whole cell and single channel responses were transient in nature, declining in the course of a few seconds. Such transient responses could, however, be observed reliably for the duration of the recordings.

These results suggest that reductions in extracellular calcium, with a magnitude and time course similar to those observed during epileptiform spikes, can induce a transient inward current. This current appears to arise from flow of sodium through modified calcium channels. Such alterations in neuronal excitability may play a role in the pathophysiology of epilepsy.

W-Pos136 SENSITIVITY OF CALCIUM CURRENTS TO ETHANOL IN APLYSIA. Patricia Camacho-Nasi and Steven N. Treistman (Intr. by Robert Weihing) Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

EtOH affects conductances underlying pacemaker activity in invertebrate neurons (Silver and Treistman, Cell. Mol. Neurobiol. 2, 1982). We extend these findings to the effects of EtOH on the calcium current (I_{Ca}) which may play a role in the modulation of pacemaker functions. Voltage clamp experiments were carried out on axotomized, identified cells of the abdominal and pleural ganglia of Aplysia californica. Isolation of I_{Ca} was accomplished by suppressing sodium conductance with TTX or Na^+-free ASW; blockade of potassium conductances was achieved by TEA and 4-AP and by holding the membrane potential at -40mV to inactivate I_A. In the majority of experiments, Ba^{++} served as charge carrier. Inward current was maximal at +5mV and latency to peak amplitude was 5.1± 2msec (mean ± sd, n=20). In all cells, EtOH produced a dose-dependent decrement in the amplitude of I_{Ca} regardless of carrier (i.e., Ca^{++} or Ba^{++}). A comparison of I_{Ca} with the other ionic currents we have examined (I_A, I_K, I_{Na}) reveals that I_{Ca} is the most sensitive to EtOH. A blockade of 20% in the amplitude of I_{Ca} is attained at 50mM EtOH. Comparable changes in amplitude (in this case, decrementing) do not occur for I_{Na} until concentrations of 200 mM (n=11) and for I_K (incrementing) until concentrations greater than 400mM (n=10). Higher concentrations of EtOH (400-600 mM) result in nearly total blockade of I_{Ca} (80-90%). This effect of EtOH on I_{Ca} amplitude is generally reversible at low concentrations, but only partially reversible at the higher concentrations. EtOH had no effect on the kinetics of activation nor was there a shift in the voltage-dependency of activation within the voltage range of -20 to +10mV.

W-Pos137 FMRF-AMIDE SUPPRESSES CALCIUM CURRENT IN APLYSIA NEURONS. V. Brezina, C. Erxleben and R. Eckert (Intr. by C. Vandenberg). Dept. of Biology, UCLA, Los Angeles, CA 90024.

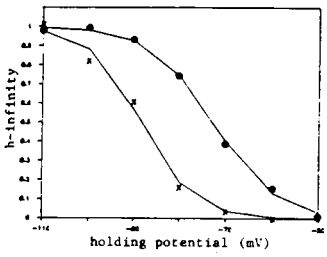
FMRFamide-like peptides found in Aplysia and other molluscan nervous systems can modify synaptic and behavioral function (Voshart and Lukowiak, 1982, Neurosci. Abstr. 8:365). We therefore examined the action of FMRFamide and YGG-FMRFamide on Ca currents in voltage-clamped Aplysia neurons. The abdominal ganglion was bathed in Na-free ASW containing 200 mM TEA and 5 mM 4-AP for virtual isolation of the Ca current. Ca (or Ba) currents were recorded during step depolarizations from -40 mV. In some of the cells examined (L7, L11, R14), the Ca current showed no response to the peptides briefly puffed onto the cell. In certain other cells (L2-L4, L6, R2, R15), however, the Ca (or Ba) current amplitude was decreased by up to 40% for 1-2 minutes following the brief application of 1 µM peptide. Ca tail currents measured near E_K showed similar reduction. The response could be elicited repeatedly with little or no desensitization, and was unaffected by injection of EGTA. The peptides produced no measurable effect on currents that remained in Ca-free TEA ASW. The percentage FMRFamide-induced decrease in Ca current remained nearly constant throughout a 100ms depolarization, except for a somewhat slower inactivation of the depressed current, as expected for Ca-mediated inactivation. In the absence of TEA, the FMRFamide-induced reduction of inward current was associated with a decrease in Ca-dependent K current (Erxleben et al., this volume). These findings cannot be accounted for by any known effects on outward currents, and appear to result from a peptidergic suppression of the Ca current. Supported by NSF BNS 83-16417 and USPHS NRSA GM07185.

W-Pos138 FMRF-AMIDE INDIRECTLY DEPRESSES Ca-DEPENDENT POTASSIUM CURRENT IN APLYSIA NEURONS. C. Erxleben, V. Brezina and R. Eckert (Intr. by G. Augustine). Department of Biology, UCLA, Los Angeles, CA 90024.

The neuropeptides FMRFamide and YGG-FMRFamide were reported to diminish the Ca-dependent K current, $I_{K(Ca)}$, in a Helix neuron by means independent of altered Ca^{2+} entry (Cottrell, 1982, Nature 296:87). In neurons L2-L4, L6, R2, R15 of Aplysia, however, $I_{K(Ca)}$ appears to be depressed by a different peptidergic mechanism. FMRFamide and YGG-FMRFamide reduce the Ca current (Brezina et al., this volume), which might secondarily depress activation of $I_{K(Ca)}$. To examine this, we puffed 1 µM of either peptide onto voltage-clamped Aplysia neurons in Na-free ASW, and recorded membrane currents in response to depolarizing steps before, and 20 s following, the puff. The peptide reduced the initial inward (Ca) current transient, and this was accompanied by a reduction of the subsequent outward (K) current. These two effects invariably occurred together. Addition of 5 mM 4-AP, which blocks Ca-insensitive K currents but not $I_{K(Ca)}$, enhanced the percentage depression of outward current. When I_{Ca} and $I_{K(Ca)}$ were both eliminated in Ca-free ASW (no 4-AP), the peptide either had no effect or it caused a small increase in a remaining outwardly rectifying K current. Ca ions were then injected in the presence of I_{Ca} blockers to evoke $I_{K(Ca)}$ transients at various potentials in the absence of a Ca current. FMRFamide had no effect on these $I_{K(Ca)}$ transients, although it still activated the outwardly rectifying K current, and had depressed $I_{K(Ca)}$ activated by Ca entry during depolarization prior to Ca current blockade. Thus, depression of $I_{K(Ca)}$ by FMRFamide in Aplysia neurons appears to result not from altered K channel properties, but instead from suppression of Ca current activation. Supported by NSF BNS 83-16417 and USPHS NRSA GM07185.

W-Pos139 A NEGATIVE VOLTAGE SHIFT IN SODIUM CURRENT INACTIVATION IN EMBRYONIC HEART CELLS CAUSED BY BATH FLOW. R. K. Ayer, Jr., S. Fujii and R.L. DeHaan (Intr. by J.R. White) Dept. of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322.

We have measured a negative shift in the voltage dependence of steady-state inactivation (h-infinity) of the sodium current (I-Na) caused by bath fluid flow in single 12-14 um embryonic chick ventricle cells at 23±1°C using the patch electrode whole cell recording (WCR) technique (Hamill et al. 1981). Cells adhering to a culture dish were bathed in static balanced salt solution (BSS) or in BSS flowing at 1 ml/min from a perfusion pump. After a 10-20 gigaohm seal was formed on a cell in static BSS the membrane patch was disrupted and whole cell clamps with series resistance less than 10 Mohms and capacitive time constants less than 100 microseconds could be obtained. The voltage dependence of h-infinity was determined by measuring peak I-Na at -20 mV activated from holding potentials between -50 and -110mV. This measurement was repeated three times in static BSS and three times again after the perfusion pump was turned on. Curves of peak I-Na versus holding potential were fit to a logistic function to obtain values of the voltage of half-inactivation (Vh). In a typical experiment (figure), Vh was constant at -73 mV for 8 min in static solution (•). After starting perfusion, Vh shifted to -84.5 mV within 1 min and to -89 mV after 8 min of constant flow (x). In 14 experiments, Vh changed by -1.0±2.1 mV during 3-8 min in static BSS, but shifted -12.4±5.9 mV 5-14 min after perfusion flow was started. (Supported by NIH HL-27385 to RLD)



W-Pos140 INTRACELLULAR NEOSTIGMINE PRODUCES PROLONGED SODIUM-DEPENDENT ACTION POTENTIALS IN RETZIUS CELLS OF THE LEECH C.N.S. W. Michael King and Charles Edwards, Department of Biological Sciences, State University of New York at Albany, Albany, NY 12222.

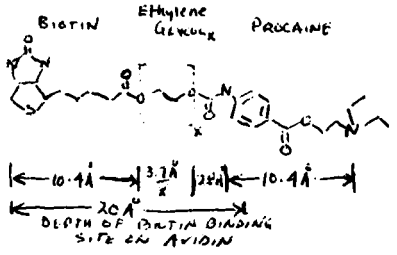
Two classes of drugs are known to prolong the action potentials of the paired giant neurons, called Retzius cells (RZ), in the segmental ganglia of the leech. The action potentials produced by both classes of drugs are similar in form and are characterized by an initial Na^+-dependent rapid depolarization followed by a sustained plateau phase that may be seconds in duration. The two classes of drugs are differentiated by the ionic dependence of the plateau phase of the prolonged action potential they produce. TEA and sparteine (Kleinhaus and Prichard, 1975, 1977; Kleinhaus, 1980) application result in action potentials with a strongly Ca^{++}-dependent plateau; whereas barbiturates, local anesthetics and physostigmine (Kleinhaus and Prichard, 1977, 1979; King et al., 1984) produced plateaus that are Na^+-dependent. The pH dependency of the effects of the latter compounds indicate that the uncharged form of each drug is the more active in action potential prolongation. Since cell membranes are permeable to the uncharged forms of these compounds, the pH dependency could reflect an enhanced access to an intracellular site of action. These considerations led us to examine the effects of neostigmine, a permanently charged impermeant analog of physostigmine. When presented in the bathing medium, neostigmine has no effect on RZ action potentials (King et al., 1984). We have found, however, that when neostigmine is applied by intracellular iontophoresis, the RZ action potential is greatly prolonged and the plateau phase of this action potential is Na^+-dependent. These results suggest the possibility that both neostigmine and physostigmine produce prolonged Na^+-dependent action potentials by acting at an intracellular site.

W-Pos141 Na CURRENTS IN CULTURED NEONATAL RAT VENTRICULAR MYOCYTES. A.E. Lacerda, D.L. Kunze and A.M. Brown, Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

The cultured neonatal rat heart ventricular myocyte (neocyte) preparation provides a good opportunity to study cardiac permeability systems with high time and current resolution through the use of the patch clamp technique. We compared the Na channel currents in neocytes as revealed by whole cell and single channel analysis, to currents of isolated adult rat ventricular myocytes. AC impedance analysis shows the neocytes to have a simple electrical structure modelled by a parallel RC circuit. A significant contribution to whole cell currents of Na channels residing in the T-tubular system can thereby be excluded. Whole cell currents were modelled by a Hodgkin and Huxley $m^3(h_1 + h_2)$ system as in the adult. Activation and inactivation time constants were similar in the adult and neocyte preparations. Recovery of the whole cell Na currents from inactivation showed a bi-exponential time course. Threshold channels of the type described by Gilly and Armstrong (Nature 309:448, 1984) were not found. At potentials just above threshold a small, very slowly inactivating current could be seen in whole cell records. A slow component was also seen in summed single channel currents at these potentials. Single channel analysis was found to be incompatible with the Aldrich, Corey and Stevens (ACS)(Nature 306:436, 1983) description of Na channel kinetics at these potentials. Neocyte Na channels in the window current region reopen much more often than ACS theory predicts. Supported by NIH grant HL25145.

W-Pos142 BLOCK OF NEURONAL NA CHANNELS BY "LEASHED" LOCAL ANESTHETICS. John Butterworth, John Moran, Gary Strichartz, George Whitesides, Anesthesia Research Laboratories, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, and Department of Chemistry, Harvard University, Cambridge, MA

In an attempt to measure the depth of the local anesthetic (LA) binding site within the neuronal membrane, LA derivatives were synthesized with a "spacer" molecule interposed between the LA and a large, impermeant macromolecule. Tetracaine and procaine were reacted though their p-NH_2 nitrogens with polyethylene glycols containing 0, 3, and 6 ethylene glycol subunits, then with D-biotin. The LA derivatives produced (T-0-Bio, P-0-Bio, P-3-Bio, P-6-Bio, respectively) inhibited the compound action potential (AP) of desheathed frog sciatic nerve in a concentration-dependent, reversible manner in the absence of equimolar avidin. Avidin bound T-0-Bio and P-6-Bio and (presumably) anchored the biotin containing end of the molecule outside the plasma membrane thereby preventing inhibition of the AP. This inhibition of LA function did not result from steric effects attendant to avidin binding since quadrupling [T-0-Bio] which produced ∿ 50% block (in the presence of avidin) did not produce comparable block in the presence of avidin. We conclude that the LA binding site is greater than 15A from the outer surface of the plasma membrane.



W-Pos143 **SODIUM CHANNEL SATURATION AND ALTERATION OF CURRENT KINETICS BY SEVERAL PERMEANT CATIONS.** Sherrill Spires, Dept. of Physiology, Univ. of Rochester, Roch., NY 14642.

Sodium channel currents of squid giant axons were studied in artificial seawater solutions (ASW) in which all external Na ions were replaced by guanidinium, formamidinium, or ammonium. Cs^+ was the only internal cation. Standard voltage clamp and internal perfusion techniques were used. There are differences in several aspects of the sodium channel current kinetics between sodium and the replacement cations. In the replacement cations: plots of steady-state inactivation (h_∞), activation, and deactivation time constants are all displaced to the right (5-15 mV) on the membrane voltage axis compared to sodium. This voltage shift is similar to that which occurs when external [Ca^{++}] is raised. In Na ASW (no Mg^{++}) a 50 mM change in [Ca^{++}] produces an 11 mV shift of the h_∞ curve, but in guanidinium ASW this same change in [Ca^{++}] produces no shift of h_∞. Thus it would appear that the replacement cations interact with the surface charges with which Ca^{++} normally interacts. However, a voltage shift cannot explain all of the differences in current kinetics: the ratio of steady-state current at the end of a depolarizing pulse to peak current is larger in the replacement cations than in sodium. To see if these differences could be related to occupancy of the Na channel by the permeating cation, peak inward current as a function of concentration of Na^+ and the replacement cations was measured. These data are described by saturating rectangular hyperbolas giving half-saturation concentrations of .33, .48, 1.09, and 1.86 M for guanidinium, formamidinium, sodium, and ammonium, respectively. Consequently, the differences in current kinetics are not correlated with channel occupancy.

W-Pos144 GATING CHARGE MOVEMENT AND Na CURRENT KINETICS OF BATRACHOTOXIN-MODIFIED Na CHANNELS. Joëlle Tanguy, Jay Z. Yeh and Toshio Narahashi, Lab. Neurobiol., École Normale Supérieure, Paris, France, and Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, Illinois 60611.

The effects of batrachotoxin (BTX) on the kinetics and steady-state properties of Na channels and their gating charge movements were studied with squid axons. After BTX modification, the channels opened at large negative potentials. The conductance-voltage relation was shifted in the hyperpolarizing direction by about 40 mV and the maximum Na conductance was only slightly affected. At potentials more negative than -50 mV, the Na current was turned on very slowly with a time constant of several milliseconds, whereas at potentials less negative than -20 mV, it was turned on with a time constant similar to that of the normal channel. Upon repolarization, the decay of Na tail current followed a double exponential time course: The fast time constant at -190 mV was about 100 μs, which was comparable to that for the control Na tail current at -90 mV. The slow component decayed with a time constant on the order of milliseconds, which was negligible in the control. Thus the kinetics of BTX-modified channel at potentials more negative than -50 mV are markedly slowed. Despite the large increase in conductance at these negative potentials, the total gating charge movement (Q_{on}) was increased only slightly over the control. This might be explained by the slow turn-on kinetics of the modified channels. At potentials more positive than -20 mV, the Q_{on} and Q_{off} were only slightly affected by BTX modification. Unlike the axon treated with pronase, the gating charge movement in the BTX-treated axon could still be immobilized even though inactivation was removed. Thus, charge immobilization does not necessarily require inactivation of the Na channel. Supported by NIH grants NS-14144 and GM-24866.

W-Pos145 SAXITOXIN INHIBITION OF SODIUM CURRENTS IN AMPHIBIAN NERVE IS INDEPENDENT OF MEMBRANE POTENTIAL. T.A. Rando and G.R. Strichartz, Anesthesia Research Laboratories, Harvard Medical School, Boston, MA 02115

Saxitoxin (STX) has recently been shown to inhibit reconstituted single Na channels in a voltage-dependent manner (Krueger, et al., Nature, 303 (1983), 172). These channels were incorporated into planar bilayers and were activated by batrachotoxin (BTX). We sought to test whether or not STX also blocked Na channels in intact nerves with a similar voltage-dependence.

Nodes of Ranvier from Rana pipiens were voltage clamped in frog Ringer, pH 7.2, containing 12 mM tetraethylammonium to block K currents. The intracellular solution contained 110 mM CsCl, 10 mM NaCl, 5 mM HEPES, pH 7.2. E_H = -130 mV, T = 23°C.

Studies of STX block of single Na channels have shown that affinity constants (K_d's) increase and relaxation time constants (τ's) decrease as the membrane potential is clamped to increasingly positive values from -80 mV. We calculated the expected change of peak Na current, I_{Na}, as a function of frequency, duration, and magnitude of depolarization. We designed pulse protocols to test for such a voltage dependence in the presence of 1.5 nM STX. Ten ms depolarizations to +50 mV delivered at 10 Hz were predicted to increase I_{Na} by 1.5x after 30 sec, 1.7x after 60 sec, and 1.9x after 90 sec. We observed that these pulse programs resulted in no change of I_{Na}. A 20 sec depolarization to +50 mV followed by a 5 sec return to E_H to recover from Na inactivation was predicted to increase I_{Na} by 3.5x. However, this also resulted in no change of I_{Na}. We conclude that the block of I_{Na} by STX does not exhibit the voltage dependence in the frog node of Ranvier that it does in single, mammalian Na channels activated by BTX.

W-Pos146 THE ACTIONS OF AN ISOLATED β-SCORPION TOXIN ON NA CHANNEL ACTIVATION. GK Wang and GR Strichartz, Anesthesia Research Laboratories, Harvard Medical School, Boston, MA 02115

Several β-scorpion toxins, isolated from Centruroides sculpturatus venom, elicit an unusual inward current after membrane depolarization in amphibian nodes. This current lasts hundreds of msecs. in the resting potential range and is totally abolished by TTX. We have used one purified β-scorpion toxin (β-C.S.IIα) on toad myelinated nerve fibers to examine 1) the voltage-dependent of the modified Na currents and 2) the "tail current" kinetics after test pulses of various duration. This toxin reduces I_{Na} during a test pulse in a voltage-dependent manner; the larger the E_{test}, the smaller the reduction in I_{Na}. Both current activation and inactivation kinetics are slowed considerably. Since P_{Na}-V curve is shifted to the depolarized direction and is less steep, the forward transitions of Na channel activation must be slowed. However, imposition of a preceding depolarizing conditioning pulse of sufficient magnitude and duration can nearly reverse the effects of toxin on P_{Na} and on the activation time course during a subsequent test pulse, although the inactivation time course is further slowed by this conditioning. In addition, the P_{Na}-V curve is shifted to the hyperpolarized direction with a maximal slope similar to the control value. This result suggests that channels activated during the conditioning pulse return slowly to the resting state so that subsequent opening requires less activation energy. The multicomponent I_{Na} tail kinetics, measured at E_H = 120 mV, are slowed progressively following a depolarization of increasing duration, as if multiple open states are present in amphibian nodes. A depolarizing conditioning pulse promotes the slowing of the tail-current decay after a following test pulse. These results are consistent with a model where Na channel activation and deactivation processes are both slowed by the toxin.

W-Pos147 INCORPORATION OF SODIUM CHANNELS FROM NORMAL AND MYOTONIC GOAT SKELETAL MUSCLE IN PLANAR LIPID BILAYERS. X. Guo, E. Moczydlowski, and S.H. Bryant, Department of Pharmacology and Cell Biophysics and Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

In a previous study using the vaseline gap voltage clamp, we found that sodium current inactivation is slower in myotonic than in normal goat skeletal muscle fibers (J. Physiol. 307: 31-32p). The present experiments were initiated to determine if this phenomenon can be correlated with abnormalities at the single channel level. Plasma membrane vesicles were isolated from normal and myotonic hind limb muscles (Biochim. Biophys. Acta. 732: 412-420) and added to the cis side of a lipid bilayer (9PE:1PC). Single Na^+ channel currents were recorded at 22°C in the presence of symmetrical 0.2 M NaCl with the cis side also containing 1.7×10^{-7} M batrachotoxin. For the normal and myotonic goat, single channel conductance was approximately 20 pS. The probability of channel opening was near 1.0 for voltages more positive than -70 mV and decreased to 0 at -120 mV as previously observed in other preparations. Block of Na^+ channels by TTX (5×10^{-8} M) was voltage-dependent with maximal block occurring at negative potentials. Batrachotoxin-activated Na^+ channels from the myotonic muscle were found to be no different than those from the normal muscle. (Supported by NIH grant NS-03178.)

W-Pos148 INTERACTIONS OF DIVALENT CATIONS WITH SINGLE SODIUM CHANNELS. Jennings F. Worley, III*, Robert J. French**, and Bruce K. Krueger*. Departments of Physiology* and Biophysics**, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Single BTX-activated sodium channels from rat brain were studied in planar bilayers containing PE (no net charge) or mixtures of PE and PS (net negative charge). Single channel currents were determined as the sodium ion concentration was altered symmetrically ($[Ca^{2+}] < 100$ nM). In both neutral and negatively charged membranes, the single channel conductance saturated at 32 pS, however, half-maximal conductance was reduced from 37 mM Na^+ in neutral membranes to 26 mM Na^+ in negatively charged bilayers (70% PS). In neutral and negatively charged membranes, extracellular Ca^{2+} produced a voltage-independent reduction in single channel currents. Similar results were obtained with Ba^{2+}, Mg^{2+}, Sr^{2+}, and Mn^{2+}. The order of efficacy is different from the affinity sequences for binding of these divalent cations to phospholipid headgroups. Potency of block by divalent cations was reduced by raising the sodium concentration, suggesting a competition between the divalent cations and sodium for a common site on the channel. Addition of trimethyloxonium tetrafluoroborate, a carboxyl modifying reagent, to the extracellular side of bilayers containing single sodium channels, eliminated STX block, reduced single channel currents 20-30%, and decreased the ability of calcium to inhibit sodium ion movement through sodium channels. These results are inconsistent with a non-specific screening effect of divalent cations, and suggest an interaction between the divalent cation and a site on the sodium channel that is capable of binding sodium, divalent cations and STX in a competitive manner. Supported by a MD. Grad. Fellowship, NIH grants NS16285 and NS20106, and U.S. Army Medical Research and Development Command.

W-Pos149 CHANNEL GATING KINETICS OF PURIFIED SODIUM CHANNELS MODIFIED BY BATRACHOTOXIN IN PLANAR LIPID BILAYERS. B. Keller*, R. Hartshorne*, J. Talvenheimo+, W. Catterall+ and M. Montal*. *University of California San Diego, La Jolla, CA 92093 and+University of Washington, Seattle, WA 98195.

Voltage dependent Na^+ channels were purified from rat brain and reconstituted in planar lipid bilayers as previously described (1,2). Here, we present the initial kinetic analysis of channel gating investigated in symmetric 0.5M NaCl-solutions in the presence of 1μM batrachotoxin (BTX). Single channel recordings were analyzed at applied voltages (V) from -110mV to -50mV (electrophysiological convention). The data were filtered at 1kHz and digitized at 200μs sampling intervals. Channel opening was voltage dependent with an apparent gating charge of ∿4. The voltage at which the channel was open 50% of the time varied from channel to channel with an average of -91 ±17mV (SD, n=22). For membrane potentials between -71mV and -95mV probability density analysis of dwell times in the open and closed states were fit well by single exponentials (to $p > 0.05$). Closing and opening rates were voltage dependent. At -85mV the average closing rate $(\text{open time})^{-1}$ was 268 $\pm 27 s^{-1}$ (SD, n=4) and it decreased with depolarization (-11 ±2mV/e-fold change). The opening rate $(\text{closed time})^{-1}$ was 162 $\pm 19 s^{-1}$ (SD, n=4) at that voltage and it increased by depolarization (13 ±3mV/e-fold change). At V more positive than -65mV, the data were not fit well by a single exponential. These properties are in agreement with those described for BTX-modified Na^+ channels in neuroblastoma cells (3). Supported by NIH, NMSS, and DAMR.

(1) Hartshorne, R. and Catterall, W. (1984) J. Biol. Chem. 259:1667-1675; (2) Hartshorne, R. et al. (1985), Proc. Natl. Acad. Sci., USA (January); (3) Huang, L. et al. (1984) Biophys. J. 45:313-321.

W-Pos150 IMMUNOAFFINITY PURIFICATION OF MUSCLE VOLTAGE-SENSITIVE SODIUM CHANNELS: ANALYSIS OF SUBUNIT COMPOSITION. J.M. Casadei, R.D. Gordon and R.L. Barchi, University of Pennsylvania, Philadelphia, PA 19104

Polyclonal antiserum (PAS) and monoclonal antibodies (MAB) raised against the voltage-sensitive sodium channel from rat sarcolemma were used to prepare immunoaffinity columns. PAS was bound to Protein A Sepharose while MAB's were covalently coupled directly to the resin (4 mg antibody/ml resin). All coupled antibodies retained their ability to specifically immunoprecipitate high affinity [^{3}H]-STX binding sites. A crude glycoprotein fraction containing the sodium channel was prepared from fresh muscle homogenized immediately in NP-40 in the presence of protease inhibitors. From this preparation MAB and PAS columns isolated a single major polypeptide that migrated as a diffuse band of 260 kd MW using SDS-PAGE. A 45 kd component was not seen; traces of a band at 38 kd were variably observed, but not in amounts stochiometrically related to the 260 kd protein. Glycoprotein fractions were also prepared in a similar manner from sarcolemmal membranes isolated from rat muscle. Proteins purified from this material by both MAB and PAS columns were analyzed by SDS-PAGE and included variable ratios of a 260 kd band and a second diffuse band of 150-200 kd, probably resulting from proteolysis of the 260 kd component during membrane isolation (Casadei et al PNAS 81:6227-31 (1984)). In addition, a 45 kd and a 38 kd MW band were seen; the 38 kd polypeptide was more prominent in sarcolemma than in the crude muscle glycoprotein fraction. We conclude that in rat muscle the sodium channel large subunit is 260 kd; the 150 kd - 200 kd band and the 45 kd band seen with conventional channel purification from isolated sarcolemma may well result from proteolysis. The functional role of the 38 kd band remains to be clarified.

W-Pos151 PURIFICATION AND FUNCTIONAL RECONSTITUTION OF THE VOLTAGE-SENSITIVE SODIUM CHANNEL FROM RABBIT T-TUBULAR MEMBRANES S.D. Kraner, J.C. Tanaka, R.H. Roberts and R.L. Barchi
University of Pennsylvania, Philadelphia, PA 19104

The voltage-sensitive sodium channel was purified from rabbit T-tubular membranes ([^{3}H]-nitrendipine binding = 41 pmol/mg, [^{3}H]-STX binding = 3.3 pmol/mg) by sequential chromatography on a guanidinium-Sepharose ion exchange column and a WGA-Sepharose lectin affinity column, followed by sedimentation on a sucrose gradient. The physical properties of the solubilized T-tubular sodium channel (Stokes radius = 8.6 nm, V = 0.83, corrected $S_{20,w}$ = 8.8 estimated protein MW = 295,000) resembled those of the rat sarcolemmal channel. Nine of 25 monoclonal antibodies raised against the rat sarcolemmal channel cross-reacted with this rabbit T-tubular channel. The purified protein contained two polypeptides of 260,000 and 38,000 MW. These proteins uniquely codistributed with the peak of [^{3}H]-STX binding and the peak of reconstitutable functional channel activity on sucrose gradients. The 260K and 38K components were separately isolated under denaturing conditions and their amino acid and carbohydrate composition determined. The purified channel was reconstituted into egg PC vesicles; the reconstituted channel was activated by BTX and Ver and blocked by STX and TTX. Cation selectivity determined quenched flow was Na^+ K^+ Rb^+ Cs^+, with $T_{\frac{1}{2}}$ uptake ratios of 1.0:0.14:0.05:0.003 respectively. Voltage-dependent activation of BTX-modified channels, determined in vesicles using rapid [K^+] jumps in the presence of valinomycin, was preserved. The rabbit T-tubular sodium channel closely resembles that purified from rat skeletal muscle and brain in its physical and functional characteristics. The 260,000 MW subunit of these mammalian channels are similar to that of the eel channel. The remaining unresolved issue is whether the small subunit(s) seen in the mammalian channels are essential for function.

W-Pos152 EFFECTS OF PHOSPHOLIPID HEADGROUP MODIFICATION IN VOLTAGE CLAMPED SQUID AXON. A. Michael Frace, *M. Poznansky, D.C. Eaton, M.S. Brodwick, Department of Physiology and Biophysics University of Texas Medical Branch, Galveston, Texas 77550; *Department of Physiology, University of Alberta, Edmonton, Alberta TG6 2E2 CANADA

We have examined the effect of phospholipid modification on Na^+ and K^+ conductance in voltage clamped squid axon. To alter phospholipid structure, headgroups were either cleaved with phospholipase or neutralized with uranyl (UO_2^{++}). Phospholipases were internally applied to continuously perfused axons. Phospholipase C (Pl C) from C. welchii and phospholipase D showed no effect. Pl C from B. cereus specifically reduced Na^+ current with little effect on K^+ current. Further isolation of a phosphatidyl inositol specific component from Pl C accentuated this response. No shifts of the conductance-voltage relationship, indicative of surface charge alteration, were evident in any phosphlipase treatments.

Uranyl, which binds avidly to phosphate groups, is known to produce large shifts in surface potential in lipid bilayers at μm concentration. We applied UO_2^{++} internally or externally in mM concentration. Biochemical assay of post experimental axons showed extensive covalent binding of UO_2^{++} or UO_2^{++}(OH) complexes to membrane lipids. UO_2^{++} caused no shift in the conductance-voltage relationship. Moreover, the shift caused by altering external calcium concentration was not affected by UO_2^{++}. Small reductions in both Na^+ and K^+ currents were observed.

This data suggests that surface potential originating from the lipid bilayer does not influence Na^+ or K^+ channel function and that phosphate groups play no role in the calcium induced IV shift in this preparation. (Supported by NIH grant NS11963).

W-Pos153 INTERACTIONS OF SPIN-LABELED LOCAL ANESTHETICS WITH RECONSTITUTED ACETYLCHOLINE RECEPTOR MEMBRANES. Julie P. Earnest, Helen Yeo, and Howard H. Wang, Department of Biology, University of California, Santa Cruz, CA 95064.

Acetylcholine receptor from **Torpedo californica**, reconstituted into bilayers of dioleoylphosphatidylcholine, were treated with spin-labeled local anesthetics under various experimental conditions. Using electron spin resonance and spectral resolution techniques (J.P. Earnest et al., Biochem. Biophys. Res. Commun. **123**: 862, 1984) the proportion of spin-labeled local anesthetic bound to the receptor was determined in the presence or absence of agonists, competitive antagonists, other noncompetitive inhibitors, and covalent modifiers of the receptor. The major population of spin-labeled local anesthetic bound to the receptor was the positively charged species. It was found that reconstituted acetylcholine receptor membranes labeled with spin-labeled local anesthetics did not show the same response to carbamylcholine as did the purified native membranes (see A. Palma **et al.**, this meeting). Other data indicated that binding of these local anesthetics to the receptor was partially prevented by preincubation with other known noncompetitive inhibitors of receptor function, such as dibucaine and phencyclidine. Alkylation of protein sulfhydryl groups also prevented local anesthetic-binding. Data is also presented which suggests that charges on both the receptor and on the lipid bilayer affect the partitioning of the local anesthetic into the membrane.

W-Pos154 LOW DENSITY LIPOPROTEIN RECEPTOR REGULATION: KINETIC MODELS: Paul W. Chun, Chan Won Lee, Rachel B. Shireman, Tae Kyung Lee, and Erich E. Brumbaugh. Dept. of Biochemistry & Molecular Biology, University of Florida, Gainesville, Florida 32610.

The macromolecular species distribution in a receptor-mediated endocytotic pathway was computer-simulated based on kinetic data reported in the literature. In the proposed model, the rapidity with which the recycled receptor is shuttled to the cell surface is indicated by the magnitude of k_{-3}, the shuttling constant. The magnitude of k_{-3} will vary with the experimental conditions, but when this value is large, the internalized receptor is shuttled back to the cell surface with a traverse time of 14 min. Under steady state conditions, after the cells have been incubated in the presence of LDL for 5 hours (Brown and Goldstein, Cell 9, 663-674, part 2, 1976), the time required for a receptor to traverse the entire endocytotic pathway is 52 min. Under these conditions, it appears that the release of the receptor from the internalized LDL-receptor complex is generally slow. In non-steady state conditions, where k_{-3} is large, the release of internalized receptor and the speed with which it is shuttled back to the cell surface are generally rapid.

Our simulation suggests that normal LDL binding in such a short-term experiment may be independent of receptor synthesis and regulation. Thus, the degradation of LDL and resultant build-up of cholesterol would have no apparent inhibitory effect on the down-regulation of receptor synthesis.

Our multi-step, irreversible ordered sequence model for the recycling of LDL receptors offers a reasonable explanation for the down-regulation of cell receptors in cultured fibroblasts under steady state conditions. (Supported by NSF PCM 81-03263 and PCM 83-12101).

W-Pos155 USE OF SYSTEMATIC STRUCTURAL VARIATION OF ANTIGENIC MOLECULES TO STUDY SIGNAL GENERATING RECEPTOR AGGREGATES IN IMMUNOCYTE MEMBRANE. Renee Z. Dintzis, Marjorie H. Middleton and Howard M. Dintzis, Dept. of Cell Biology and Anatomy and Dept. of Biophysics, Johns Hopkins Medical School, Baltimore, Maryland 21205.

Certain polymeric molecules with repeating identical haptenic groups are capable of stimulating a B lymphocyte to differentiate into a plasma cell producing antibodies against the hapten. This is believed to occur via a signal generated by the cross-linking of immunoglobulin receptors in the B lymphocyte membrane. However, not all molecules fitting the above description can generate an immunogenic signal; indeed, some can inhibit signal generation. We have synthesized a series of hapten-substituted polymeric molecules, systematically varying their molecular weights and hapten numbers, and have studied their abilities to stimulate or inhibit the formation of immunogenic signals. Knowledge of the molecular properties of such polymers gives valuable clues as to the size of the membrane receptor aggregate necessary for signal generation. Signal generation was measured by in vitro enumeration of antibody-forming cells (plaque assay) and by in vivo determination of serum antibody concentration (modified radioimmunoassay). Using linear polyacrylamide molecules substituted with the dinitrophenyl (Dnp) hapten, it was determined that only those molecules with M.W. over 100,000 and with 20 or more Dnp groups could form a threshold receptor aggregate large enough for signal generation. Inhibitory smaller molecules with high hapten density could prevent formation of large receptor aggregates by competing for receptors. At high doses, even immunogenic molecules could inhibit threshold-sized receptor aggregates, their inhibitory abilities correlating with their hapten density.

W-Pos156 MECHANICAL RESPONSE OF COCHLEAR OUTER HAIR CELLS TO TRANSCELLULAR STIMULATION WITH ALTERNATING CURRENTS. B. Kachar, W. E. Brownell, J. Fex, and R. Altschuler, NINCDS-NIH, Bethesda, Md. 20205, Univ. of Florida, Gainesville, FL 32610. (Intr. by J. E. Zengel).

100 µl aliquots of culture medium containing isolated outer hair cells (OHC), obtained after gentle mechanical dissociation of guinea pig cochlea, were placed on a polylysine coated microscope slides. OHCs attached to the glass surface at either their synaptic or stereocilia ends. A pair of silver wire electrodes, separated by a distance of 100-150 µm, were mounted to the condenser of an inverted microscope equipped for video contrast enhancement microscopy. Sinusoidal currents of 5-10 Hz and less than 1 mA were passed across the length of the cell. Cell response to the electric stimulation was analyzed from slow motion and frame by frame play back of recorded experiments. Stimulus evoked displacements of the cell's free end with magnitudes between 0.2-1 µm could be visualized at the high resolution of the video images. Measurements taken from the TV screen were plotted on graphs and analyzed. Cell length was sinusoidally modulated about its resting value at the stimulus frequency. There was little evidence of rectification or harmonic distortion. Motility was not observed in other cells (red blood cells, Deiter and Henson cells) that coisolate with the outer hair cells. Stimulus related movements of stereocilia were also not observed. OHC responses were unaffected by the presence of 2 mM DNP or 200 µg/ml of IAA acid (metabolic uncouplers). The time course, bidirectionality, and independence of ATP argue against a contractile mechanism, but are compatible with electro-kinetic processes. Special features of OHCs such as their elongated shape and the presence of laminated cisternae, may provide the morphological substrate for electro-osmotic-like phenomena. A direct electrophoretic effect on surface charges, however, cannot be excluded. Electrokinetic mechanisms are attractive because they may permit mechanical responses of the short time constants required to match auditory frequency responses.

W-Pos157 EQUILIBRIUM AND KINETIC PARAMETERS FOR ANTIBODY-HAPTEN INTERACTIONS IN A LIPOSOMAL MODEL SYSTEM. Ashot Petrossian and John C. Owicki, Dept. of Biophysics & Medical Physics, Univ. of CA, and Div. of Biology & Medicine, Lawrence Berkeley Labs, Berkeley, CA 94720.

Recently we described qualitatively the interactions of monoclonal IgG and Fab with a hapten, fluorescein, that is conjugated to a lipid and incorporated into liposomes (Petrossian and Owicki (1984) Biochim. Biophys. Acta, 776:212-227). The hapten existed in two conformations: extended away from the membrane (available for antibody binding) and sequestered at the membrane surface (unavailable for binding). Here we report the quantitative analysis of equilibrium and kinetic parameters for the interactions. These are obtained by using non-linear least-squares algorithms to fit experimental data to the following system of mass-action expressions:

$$H_s \underset{k_{-0}}{\overset{k_0}{\rightleftharpoons}} H_e \qquad F_{ab} + H_e \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} F_{ab}{\cdot}H \qquad IgG + H_e \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} IgG{\cdot}H \qquad IgG{\cdot}H + H_e \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} IgG{\cdot}H_2$$

For liposomes containing 0.1 mole % hapten and the balance various phosphatidylcholines (PC) or PC/cholesterol mixtures, the following results obtained: k_0 and k_{-0} depended highly on lipid composition and were no larger than 0.01/sec. The equilib. const. $K_0 = k_0/k_{-0}$ ranged from 0.02 to 3. F_{ab} bound with an affinity $K_1 = k_1/k_{-1}$ close to that for Na fluorescein (NaF; 0.4/nM), and k_1 was no slower than half that for NaF (7/µM/sec). $K_2 = k_2/k_{-2}$ was effectively much smaller than K_1, but considerable bivalent binding occurred nevertheless. K_2 varied directly with the lateral density of hapten. IgG dissociation from liposomes, measured by competition with a nonfluorescent hapten analog, was up to 50 times slower than from NaF. Support: NIH #R01-AI-19605-02.

W-Pos158 ELECTROPHYSIOLOGICAL STUDY OF ANGIOTENSIN II ACTION ON ISOLATED RAT ADRENAL GLOMERULOSA CELLS. S.J. Quinn, M.C. Cornwall and G.H. Williams*, Department of Physiology, Boston University School of Medicine, Boston, MA 02118 and *Endocrine-Hypertension Unit and Department of Medicine, Brigham and Women's Hosptial, Boston, MA 02115.

Angiotensin II is a well known secretagogue for the production of aldosterone by adrenal glomerulosa cells. The electrophysiological effects of angiotensin II on isolated rat glomerulosa cells were examined using intracellular voltage recordings. At resting membrane potential, angiotensin II(10^{-11}M) produced a biphasic membrane response: a brief, initial hyperpolarization associated with a conductance increase and a second, long-lasting depolarization due to a conductance decrease. These membrane responses were dose-dependent over the same concentration range which stimulates aldosterone production in secretion studies on the same preparation (Braley and Williams, 1977).[1] The angiotensin II effects were sustained following substitution of external Ca^{++} by Ba^{++} and Sr^{++}, but were inhibited by addition of Mn^{++} or the removal of external Ca^{++}. The reversal potential for both phases was -95mV in solution containing 4mM K^+ and changed linearly with the log of $[K^+]_o$. The slope of this linear relationship was 58mV/ten-fold change in $[K^+]_o$. These data indicate that angiotensin II elicits a dose-dependent change in potassium conductance. In addition, several voltage-dependent membrane potential changes were sensitive to angiotensin II. When depolarizing current pulses were applied in the presence of angiotensin II, the Ca^{++} action potential and afterhyperpolarization noramlly observed in these cells were inhibited. Supported by BUSM-GSRA Award # X20.

1. L.M. Braley and G.H. Williams, Amer. J. Physiol. 233, E402-E406, 1977.

W-Pos159 cAMP AND BASOLATERAL K EFFECTS ON PEAK AND STEADY-STATE SODIUM CURRENT KINETICS IN R.PIPIENS SKIN. Robert A.Grossman and T.Hoshiko, Dept. of Physiology, Case Western Reserve University, Cleveland OH, 44106, USA

In order to test the conjecture that serosal K effects are mediated via cAMP release, their effects on Michaelis-Menten-type Na-Isc parameters were compared in 14 skins. Skins were presented with 3,4,6,12,24,48,80 or 120 mM apical Na in succession followed by 100 μM amiloride in that Na conc. and a 15 min. recovery in 0.1mM Na. The peak and steady-state (at 3 min) amiloride-sensitive Isc were used to estimate Imax and Km then again after bathing the corium in 4 mM cAMP for 1 hour. The whole procedure was repeated with serosal K as the experimental treatment. In paired comparisons, both Km and Imax measured for the peak currents were significantly higher than for the steady-state parameters. Km for the peak response in both cAMP and serosal K increased significantly. Km in the steady-state was significantly higher after cAMP but after serosal K, just missed significance, i.e., a larger series should show significance. Imax was higher also but significant only for peak and steady-state responses after cAMP, a not unexpected result since Isc after serosal K was smaller and more variable. In conclusion, serosal K effects though smaller in magnitude are similar to those of cAMP and the results are consistent with the initial conjecture. (Supported by grant AM 08565).

W-Pos160 CELL MEMBRANE CATION SELECTIVITY OF CULTURED TOAD KIDNEY CELLS (A6) STUDIED WITH INTRACELLULAR MICROELECTRODES. S.R. Thomas and E. Mintz. INSERM U.192, Hôpital Necker, 75743 Paris, France and CEN Saclay, Département Biologie, 91191 Gif sur Yvette Cedex, France.

Intracellular microelectrodes were used to study cation selectivity of apical and basolateral cell membranes of A6 cells grown on collagen-coated polycarbonate filters in cups with "feet". Chamber design permitted several solution changes during a given impalement. In "young" cultures, i.e. before the development of transepithelial PD, V_{ms}, mucosal replacement of Na^+ by K^+ caused dramatic depolarization of apical PD, V_{mc} (> 40 mV fall), suggesting a K-selective membrane. Amiloride (10^{-4}M) had no effect on either V_{mc} or transepithelial resistance, R_{ms}, in these tissues. In "mature" cultures (10-20 days), V_{ms} (11.7±2.2 mV) and R_{ms} (2.8±0.2 kΩcm^2) are stable and the apical cell membranes become highly selective for Na^+: total mucosal replacement of Na^+ by either K^+ or TEA^+ results in 10 mV hyperpolarization of V_{mc} (9.33±0.33 and 10.1±1.1 mV, resp.), as well as increased voltage divider ratio, F, from 0.81±0.03 to 0.95±0.006 (data pooled). R_{ms} increased 52±10% with K^+ and 16±6% with TEA^+ as Na^+ replacement. Mucosal amiloride ($5x10^{-5}$M) in normal Ringer hyperpolarized V_{mc} and increased F to the same extent as the ionic replacements, increased R_{ms} by 16±5%, and reduced V_{ms} to < 2 mV or short-current to near zero. Even in these mature, apically-Na-selective cells, serosal addition of high $[K^+]$ depolarized V_{mc} by > 40 mV, confirming the apical/basolateral polarization characteristic of other "tight" epithelia. Based on our measurements in symmetrical solutions, a circuit analysis assuming amiloride affects only apical membrane resistance (but not necessarily total block of cell pathway) permitted calculation of apical, R_a, basolateral, R_b, and shunt resistances, R_s : in kΩcm^2, R_a = 12.1 ± 1.7, R_b = 2.5 ± 0.5, R_s = 3.4 ± 0.5 and after amiloride, R_a = 47.3 ± 0.03.

W-Pos161 CELL Na ACTIVITY TRANSIENTS IN FROG SKIN DURING INHIBITION OF CELLULAR CURRENT. J.F. García-Díaz, L.M. Baxendale and A. Essig. Department of Physiology. Boston University School of Medicine, Boston, Massachusetts.

Cell Na activity (a^c_{Na}) was measured in the short-circuited frog skin (R. pipiens pipiens) by simultaneous impalements from the apical surface with single-barreled open-tip (1.5M KCl, 40-80 MΩ in Ringer's) and Na-selective (Neutral Ligand I, Fluka) microelectrodes. The Na-selective microelectrodes were calibrated in Ringer and in solutions of variable [Na] with [K] ∿ 117 mM and pCa = 7.0. These electrodes responded by less than 1 mV to changes in pCa from 8 to 6. Cell current (I_c), ranging between 20 and 30 μA/cm^2 with apical [Na] 110 mM, was abolished either by addition of amiloride (20 μm) or removal of apical Na (N-methyl-D-glucamine or tetramethylammonium substitution).

Addition of amiloride decreased a^c_{Na} from 8.8 ± 1.0 mM (SD, n=4) to 6.1 ± 0.6 mM in 3 min and to 3.3 ± 1.2 mM (n=2) in 9 min. Removal of Na decreased a^c_{Na} from 10.2 ± 0.9 mM (n=3) to 5.5 ± 0.9 mM in 3 min and to 2.5 ± 1.6 mM (n=2) in 9 min. Restoration of apical Na returned I_c to its initial value in < 30 sec and increased a^c_{Na} from 2.8 ± 1.6 mM (n=5) to 8.5 ± 1.6 mM in ∿ 5 min. These increases in a^c_{Na} were faster than the decreases after Na removal; a^c_{Na} attained half the final value in ∿ 50 - 100 sec. Assuming constancy of cell volume during this period, the basolateral Na current (I^b_{Na}) was calculated from $I^b_{Na} = I_c - \mathcal{F} \cdot h/\gamma \, (da^c_{Na}/dt)$, where h is the assumed effective cell length (∿ 45μm) and γ the cell activity coefficient for Na (∿ 0.77). I^b_{Na} increased hyperbolically with a mean $t_{1/2}$ of ∿ 40 sec, reaching 0.95 I_c in ∿ 5 min. [Supported by USPHS grant AM 29968 and (L.M.B.) USPHS Training Grant T37 AM 07053].

W-Pos162 **SINGLE-CHANNEL CURRENTS RECORDED FROM ISOLATED TURTLE COLON EPITHELIAL CELLS.** Neil W. Richards and David C. Dawson, (sponsored by James H. Sherman), Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

The single-channel recording technique has been applied to a variety of cell types including epithelial cells. We report here the application of this technique to isolated epithelial cells from the turtle colon. Single epithelial cells were isolated by incubating mucosal scrapings with hyaluronidase (1 mg/ml) for 20 minutes. Patch-clamp pipettes were fabricated as described by Hamill et al. (Pflugers Archiv. 391:85, 1981) using either boralex micropipettes or Corning #7052 glass as described by Rae and Levis (Mol. Physiol. 6:115, 1984). Although it was possible to form giga-ohm seals on isolated epithelial cell membranes using either glass, seals fromed more readily with Corning #7052. Using such pipettes, we have identified at least two types of unitary membrane conductance. When cells were bathed in 112 mM K Ringer's to depolarize the resting membrane potential [with Na Ringer's (K=7.5 mM) in the pipette] we recorded unitary events with conductances of 18 -20 pS and apparent reversal potentials of 50-70 mV, cell interior negative. These properties are those expected for channels selective for K. In detached patches (inside-out) with a variety of solutions in the bath and the pipette we have recorded a 30 pS channel which could be either selective for chloride or non-selective for cations. These results indicate that single-channel recording methods can be applied to freshly dissociated colonic epithelial cells. (NIH support)

W-Pos163 **CALCIUM ACTIVATION OF A BASOLATERAL K CONDUCTANCE IN DIGITONIN-PERMEABILIZED EPITHELIAL CELLS.** Dean Chang and David C. Dawson, Department of Physiology, University of Michigan Ann Arbor, MI 48109.

In turtle colon epithelial cells osmotic swelling activates a quinidine-sensitive basolateral K conductance (Fed. Proc. 43:892, 1984). We investigated the possible role of intracellular calcium as an activator of basolateral K conductance by using digitonin (20 μM) to permeabilize the apical membrane. Digitonin treatment is thought to result in equilibration of cytosolic and extracellular calcium. Portions of stripped turtle colon were mounted in Ussing chambers. The serosal solution was Na Aspartate Ringer's containing 1 mM calcium. The mucosal solution was K Aspartate Ringer's which contained sufficient EGTA to reduce the concentration of ionized calcium to 1 nM to 10 μM. Transmural K currents were measured by voltage clamping the transepithelial P.D. to zero. In the absence of digitonin I_K was near zero due to the small K conductance of the apical membranes. The effect of digitonin on I_K was highly dependent on the mucosal ionized calcium concentration. When mucosal ionized calcium was 1 nM digitonin, addition increased tissue conductance but did not affect I_{sc}. In contrast, when mucosal ionized calcium was about 1 μM digitonin induced a steady-state I_{sc} which was inhibited by quinidine. In tissues exposed to digitonin in the presence of 1 nM ionized calcium, raising mucosal ionized calcium to 1 μM resulted in the prompt development of a I_{sc} which was blocked by quinidine. Reversing the orientation of transepithelial K emf reversed the direction of the calcium-activated current. These observations are consistent with the notion that changes in intracellular ionized calcium are important in the regulation of basolateral K conductance in epithelial cells. (NIH support)

W-Pos164 SINGLE CHANNEL CONDUCTANCE OF DOG TRACHEAL (CULTURED) EPITHELIAL CELLS. Jerry Farley, Terry Dwyer, Ray Frizzell, and Richard Shoemaker. Depts. of Pharmacology and Toxicology and Physiology and Biophysics, Univ. MS Med. Ctr., Jackson, MS 39216, and Dept. of Physiology and Biophysics, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Musocal strips of the dog tracheal lumen were minced and incubated for 20 minutes, 5°C, in HEPES-Ringer containing antibiotics; then the tissue was transferred to similar solution containing 1% Type 1 Collagen, room temp, and the supernatant was removed at 30 min intervals and stored in cold DME/F12 medium containing 5% F.B.S. and 1mM dithiothreitol. The isolated cells were plated on collagen coated plastic cover slips and grown in DME/F12 (50/50) medium containing: 5% F.B.S., 1% gentamycin, and 2% penstrep. The cells were refed every three days. Eight to 16 days after plating, the cells in a non-confluent area of a cover slip were used. The Na^+, K^+ and Cl^- concentrations were varied to help identify and characterize the channel types. Experiments were conducted at 11°C. Seal resistances of the patches were greater than 10 GΩ and the patches were stable for 15 to 45 min. The channels from 18 inside-out patches were analyzed. The three most prevalent channels were: (1) K^+ channels with a conductance (g) of about 50 pS; (2) Cl^- channels that had a conductance ∿ 15 pS; (3) Cl^- channels with a g > 250 pS (peak g was measured at 380 pS). This large Cl^- channel could possibly account for the large increase in conductance in the in vitro trachea following β-adrenergic stimulation. (Research support from: Ala. C. F. Foundation, NS 17789; DAMD 17-83-C-3248.)

W-Pos165 THE UNSTIRRED LAYER IN INTESTINAL ABSORPTION John A. DeSimone, Department of Physiology and Biophysics, Medical College of Virginia, Richmond, Va. 23298

When segments of small intestine are perfused with solutions containing nutrient precursors such as disacharides and pepides, hydrolysis by brush border enzymes may be diffusion controlled. The extent to which hydrolysis is diffusion-controlled can be estimated by comparing the K_m values for hydrolysis in situ with that obtained for the enzyme in free solution. In general the greater the difference, the more the in situ process is diffusion-controlled. The difference in K_m values is often used to compute the so-called "unstirred layer thickness". The number so calculated is usually considered to be of uniform thickness along the intestinal length and its relation to the prevailing hydrodynamic conditions is unspecified. I present an analysis in which the equations of laminar flow and convective-diffusion are solved in both the radial and axial coorldinates. I derive the dependence of the unstirred layer thickness on the axial position along the intestinal length for a diffusion-controlled reaction. As expected the unstirred layer thickness grows as the reaction depletes the substrate from proximal to distal. The unstirred layer thickness at any point along the intestine is inversely proportional to fluid velocity. Using data from the literature I show that 50% of the hydrolysis of sucrose occurs in the first 32% of the intestinal segment where the unstirred layer thickness is less than the mean value. Thus the average unstirred layer thickness found by conventional methods gives a misleading perception of the actual reaction dynamics. I present methods for calculating the degree to which a brush border reaction is diffusion-controlled. Two reactions for which the mean unstirred layer thickness is the same, may still differ considerably in the extent to which each is diffusion controlled.

W-Pos166 NETWORK THERMODYNAMIC MODELING OF INSULIN AND ALDOSTERONE STIMULATION OF Na^+ TRANSPORT IN CULTURED RENAL EPITHELIUM. Mark L. Fidelman and Donald C. Mikulecky, Department of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298.

A Network Thermodynamic model has been developed to describe ion flows (Na^+, K^+, Cl^-) and related electrical events in a well-defined renal epithelium in continuous culture (A6). The model consists of four membranes (apical, basolateral, tight junction, and basement) consistant with epithelial morphology. Transport of ions across individual membranes is described by either saturable channels (apical Na^+ and basolateral K^+) or simple electrochemical diffusion. The number of basolateral K^+ channels is regulated via a feedback relationship with intracellular K^+. Na-K pumps (fixed 3:2 stoichiometry) are located in the basolateral membrane and there is a fixed anion charge within the cell. Electroneutrality is imposed on all compartments.

Proposed mechanisms of action for the regulation of Na^+ transport by insulin and aldosterone, both seperately and in combination, were examined using the model and were found, in general, to be inadequate in accounting for observed behavior. In order to produce "reasonable" solutions that were consistant with experimental observations and basic thermodynamic constraints, such as electroneutrality and osmotic equilibrium, the following requirements were found to be necessary: (1) that stimulation of active Na^+ transport could be produced only with strict coordination of changes in transport parameters at both the apical and basolateral membranes; and (2) that tight junction permeability increase proportionally with increasing active Na^+ transport. With respect to (1), these simulations demonstrate that changes in transport parameters at only one membrane (either apical or basolateral) cannot by themselves produce "reasonable" solutions.

W-Pos167 EFFECTS OF TRANSPORT INHIBITION ON CELL VOLUME IN FROG URINARY BLADDER. C. William Davis and Arthur L. Finn, Depts. of Physiology and Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Cell volume in transporting epithelial cells is at a steady-state when the net solute fluxes across the apical and basolateral membranes are equal. To examine possible control mechanisms operative at the basolateral membrane of frog urinary bladder, we suddenly inhibited the Na influx across the apical membrane with amiloride, or the activity of the Na/K pump in the basolateral membrane with ouabain, and determined the resulting effects on cell volume. In the absence of secondary, or compensatory, responses, the cells would be expected to shrink following amiloride inhibition and to swell after ouabain inhibition. Cell volume, as determined by quantitative microscopy, was unchanged following mucosal application of amiloride (0.1 mM): 15 min post-amiloride, cell volume was 99.4 +/- 2.3 % of control (n=6). Serosal application of ouabain (0.1 mM) resulted in cell shrinkage: 12 min post-ouabain, cell volume was 81.1 +/- 5.5 % of control (n=4). These results indicate that secondary changes in membrane permeability accompany transport inhibition. One possible mechanism is a basolateral membrane Ca-sensitive K permeability; we therefore studied the effects of ouabain in the absence of serosal Ca. Ouabain caused the cells to swell in this circumstance: cell volume at 10 min was 132 +/- 8.4 % of control (n=4). These results are consistent with a basolateral membrane K permeability that is controlled by intracellular Ca, the level of which is sensitive to intracellular Na. A central role for Ca in the control of the basolateral membrane function is thereby indicated, and a Na/Ca exchange mechanism is implicated in the modulation of Ca levels. Supported by NIH grant AM25483.

W-Pos168 SINGLE CHANNEL IONIC CURRENTS IN PANCREATIC BETA CELLS. J.L. Schwartz*, G.A.R. Mealing*, M.D. Payet+, E.C. Rousseau+ and R. Sauvé#, *Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada, +Department of Biophysics, Faculty of Medicine, University of Sherbrooke, Canada, and #Department of Physiology, University of Montreal, Montreal, Canada.

Discrete current levels were recorded from neonatal rat beta cells in culture using the patch clamp technique. In the cell-attached configuration with high potassium in the pipette, the cells were found to be permeable to potassium. The potassium conductance was linear in the physiological range of applied membrane potentials. Its value was around 37pS. Other preliminary results are presented, which show the sensitivity of this potassium conductance to calcium and glucose concentration changes. It is postulated that this conductance is a $g_K(Ca)$.

W-Pos169 GLUCOSE-INDUCED OSCILLATORY CHANGES IN INTERSTITIAL K CONCENTRATION $(K^+)_{IS}$ IN MOUSE ISLETS OF LANGERHANS. E.M. Perez-Armendariz. and I. Atwater. Biophysics Dept., UEA, Norwich, England. N.I. A. D.D. K., N. I. H ., Bldg. 4, Rm. 312, Bethesda, MD. 20205, U. S. A.

During glucose perfusion (11 mM), pancreatic B cells (BC) show membrane potential oscillations between two levels: -45 mV (silent phase) and -35 mV (active phase (a. ph.)). A burst of Ca^{++}-spikes is generated during the a.ph. This behavior has been suggested to be due to changes in - $(Ca^{++})_i$-dependent K^+ permeability (Pk- $(Ca^{++})_i$) (1). We measured $(K^+)_{is}$ in the presence and absence of glucose using K^+ sensitive microelectrodes prepared with Corning 477317 resin. Experiments were conducted on isolated islets of Langerhans from mouse pancreas.

Intracellular recordings from BC simultaneous to K^+ recordings in the interstitial space showed each burst of electrical activity accompanied by an increasing $(K^+)_{is}$ along the a.ph, reaching a maximum of about 1.0 mM above basal $(K^+)_{is}$. An increase in the external calcium concentration (3x) produced a 33 % increase in $(K^+)_{is}$ max. It is proposed that the space between B-cells constitutes a restricted diffusion system where potassium accumulates during the activation of voltage gated and $(Ca^{++})_i$-dependent potassium channels with each burst of electrical activity.

(1) Atwater et al. J. Horm. Metab. Res. Suppl. Series 10: 101, 1980.

W-Pos170 IONIC CURRENTS AND CHANNELS IN PEPTIDERGIC NEUROSECRETORY TERMINALS. J.R. Lemos, E.L. Stuenkel, J.J. Nordmann & I.M. Cooke. Bekesy Laboratory of Neurobiology, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822.

The sinus gland, a neurohemal organ of crustaceans, is a dense aggregation of peptidergic neurosecretory terminals. In certain crabs, e.g. Cardisoma carnifex, terminals are large enough for intracellular recording (for refs. see Stuenkel, J. Physiol., in press). We now report that terminals are easily isolated following gentle collagenase treatment. In EM they appear as circular profiles densely packed with neurosecretory granules. A single sinus gland yields hundreds of isolated terminals >10 μm in dia. These terminals readily form seals (>10 Gohm) with patch clamp electrodes. Under voltage clamp, following rupture of the membrane patch ("whole terminal recording": WTR), terminals display a small (13-50 pA), rapidly activating but slowly inactivating inward current upon depolarization. This current has the expected properties of a Ca current: it is not blocked by TTX and "runs down" after 30-40 min. Outward currents were blocked by TEA.

Single channel currents were recorded from terminals in cell-attached and inside-out patches under conditions favoring K-channels (300 mM KCl on both sides of the patch). Two species of channels have been observed: 1) A slowly activating outward current channel was observed in response to large voltage steps (±50 mV from rest) with intracellular $[Ca^{++}]$ = 10 nM. This channel has a conductance of ca. 70 pS, and, in inside-out patches, is activated by increasing $[Ca^{++}]$ and blocked by TEA. It resembles, especially in its kinetics, the Ca-activated K channel seen in secretory cells from kidney tubules. 2) A rapidly activating and inactivating outward current channel which is not blocked by TEA and has a unit conductance of 7-10 pS. Other possible channels (e.g. Ca, Na, Cl) are currently being investigated in these terminals.

W-Pos171 LIGHT SCATTERING CHANGES ASSOCIATED WITH SECRETION FROM NERVE TERMINALS OF THE MAMMALIAN NEUROHYPOPHYSIS ARE DEPRESSED BY AMINOGLYCOSIDE ANTIBIOTICS. T.D. Parsons, A.L. Obaid and B.M. Salzberg (University of Pennsylvania).

Light scattering changes recorded from the terminals of the neurohypophysis of the CD-1 mouse monitor events associated with neurosecretion. The aminoglycosides are a class of antibiotics that may exhibit ototoxicity and neuromuscular blockade as clinical side effects, and animal studies of the neuromuscular junction have shown that these drugs depress evoked transmitter release by competing with $[Ca^{++}]_o$. Low concentrations of aminoglycoside antibiotics reversibly depressed the magnitude of this light scattering signal. A sixty percent decrease was observed following an 11 minute exposure of the preparation to a 220 μM concentration of neomycin. The light scattering signal responded to neomycin in a dose-dependent manner. Plots of the logarithm of the change in light scattering vs. the logarithm of the $[Ca^{++}]_o$ indicated that neomycin (22 μM) behaves as a competitive inhibitor of $[Ca^{++}]_o$ when calcium was varied from 0.5 mM to 10 mM.

Gentamicin, which is commonly employed in tissue culture media at 100 ug/ml, also depressed the intrinsic optical change. This concentration reversibly decreased the light scattering signal by nearly 40% after 11 minutes exposure. The effects of aminoglycoside antibiotics on an intrinsic optical change correlated with secretion at vertebrate nerve terminals provide additional evidence that these agents act presynaptically. Moreover, the compatibility of our results with data obtained from the neuromuscular junction reinforces the interpretation that these light scattering changes reflect processes closely related to neuropeptide release. Supported by USPHS grant NS 16824 and a VMSTP fellowship to TDP.

W-Pos172 ELECTRICAL PROPERTIES OF ISOLATED RAT ADRENOCORTICAL CELLS. M.C. Cornwall, S.J. Quinn, and G.H. Williams*. Dept. of Physiology, Boston Univ. School of Medicine, Boston, MA 02118 and *Endocrine-Hypertension Unit and Dept. of Med. Brigham & Women's Hosp. Boston, MA 02115.

Adrenocortical cells secrete steroid hormones in response to specific secretagogues. Transmembrane ion fluxes have been implicated in secretagogue-mediated steroidogenesis, but little is known of the electrical properties of these cells. Isolated rat glomerulosa and fasciculata cells were examined using intracellular voltage measurements. Average resting membrane potential in glomerulosa cells was -78 mV; that in fasciculata cells was -75mV. At rest, both cell types were relatively impermeable to Cl^- and Ca^{++} and were well-described by a Goldman-Hodgkin-Katz relation for membrane permeability to Na^+ and K^+. P_{Na}/P_K was estimated to be 0.02, while the $[K^+]_i$ ranged from 140-178mM. Depolarizing current pulses applied through the recording microelectrode elicited action potentials followed by afterhyperpolarization in both cell types. The threshold potential required to elicit the action potential averaged -65mV for glomerulosa cells and -50mV for fasciculata cells. Using the maximum rate-of-rise as an approximation of th early current during the action potential, the membrane potential for 50% inactivation of the voltage-dependent conductance was estimated to be -80mV for glomerulosa cells and -65mV for fasciculata cells. This transient response retained its waveform and amplitude in Na^{++}-free solution, but was abolished in zero external Ca^{++}. The action potential was sustained following Ca^{++} replacement by Ba^{++} and Sr^{++} and is inhibited by the addition of Mn^{++} and Co^{++}. Both the peak amplitude and maximum rate-of-rise increased linearly with the log of $[Ca^{++}]_o$. These data are consistent with the existence of voltage-dependent calcium conductance in both adrenal glomerulosa and fasciculata cells.