M-PM-Po43 LATERAL DIFFUSION OF GRAMICIDIN C IN PHOSPHOLIPID MULTIBILAYERS CONTAINING 0-50 MOLE% CHOLESTEROL. D. W. Tank[+*], E. S. Wu[+§], P. Meers[+] and W. W. Webb[+] (Intr. by E. L. Gasteiger) [+]Cornell University, Ithaca, NY 14853, [§]University of Maryland Baltimore County, Baltimore, MD 21228.

The antibiotic gramicidin C dimerizes to form a transmembrane channel in lipid bilayer membranes and has served to model ion transport in biomembranes. By the technique of Fluorescence Photobleaching Recovery, we have measured the lateral diffusion coefficient (D) of active, dansyl labeled gramicidin C (DGC), under conditions in which the cylindrical dimer molecule predominates. In pure dimyristoyl phosphotidylcholine (DMPC) bilayers, D decreases from $6.10^{-8}cm^2/sec$ at 40°C to $3.10^{-8}cm^2/sec$ at 25°C and dropped 100 fold at 23°C, the phase transition temperature (T_m) of DMPC. Above T_m, addition of cholesterol decreases D; a 3 fold stepwise drop occurs between 10 and 20 mole%. Below T_m, increasing cholesterol increases D; a 10 fold increase occurs between 10 and 20 mole% at 21°C, between 20 and 25 mole% at 15°C, and between 25 and 30 mole% at 5°C. In egg phosphotidylcholine (EPC) membranes, D decreases from $5.10^{-8}cm^2/sec$ at 35°C to $2.10^{-8}cm^2/sec$ at 5°C; addition of equimolar cholesterol reduces D by a factor of 2. The effect of gramicidin C (GC) concentration on the lateral diffusion of DGC and the fluorescence labeled lipid probe NBD-PE was also studied. Increasing GC in DMPC/GC multibilayers lowered T_m and broadened the transition. The diffusion coefficient of NBD-PE at 30°C decreases from $8.10^{-8}cm^2/sec$ below 5 mole% GC to $2.10^{-8}cm^2/sec$ at 15 mole% GC, and D for DGC similarly decreases from $4.10^{-8}cm^2/sec$ at 2 mole% GC to $1.4.10^{-8}cm^2/sec$ at 15 mole% GC. These results provide measures of possible protein-lipid and protein-protein interactions, and test the Saffman-Delbruck theory of diffusion.

M-PM-Po44 LATERAL DIFFUSION OF GLYCOPHORIN RECONSTITUTED INTO PHOSPHOLIPID MULTIBILAYERS. E. S. Wu[+§], P. S. Low[*‡] and W. W. Webb[+], [+]Cornell University, Ithaca, NY 14853, [§]University of Maryland Baltimore County, Baltimore, MD 21228, and [‡]Purdue University, West Lafayette, IN 47907.

Glycophorin is the major integral glycoprotein in human erythrocyte membranes. It spans the membrane with a hydrophobic intramembranous section and large extramembranous portions on each side. Purified glycophorin (M.W.=31,000) has been labeled with fluorescein amine by a technique that is specific for sialic acid residues in the NH_2-terminal region. Labeled glycophorin was reconstituted in dimyristoyl phosphotidylcholine (DMPC) and egg phosphotidylcholine (EPC) multibilayers. The lateral diffusion coefficients (D) were measured by Fluorescence Photobleaching Recovery (FPR). In DMPC membranes, $D=1.7x10^{-8}cm^2/sec$ at 24°C. D drops by two orders of magnitude at the phase transition between 23.8 and 23°C. Below 23°C, $D \leq 10^{-10}cm^2/sec$ but the fluorescence recovery remains close to 100%. The sharp transition between 23.8 and 23°C was eliminated by adding 50% (mole) cholesterol in DMPC. $D=2x10^{-8}cm^2/sec$ for glycophorin in EPC membranes at 24°C, increasing by a factor of 2 at 40°C. Glycophorin did not affect the diffusion of a fluorescence labeled lipid NBD-PE, which remained near its normal value $\sim 7x10^{-8}cm^2/sec$ at lipid/protein = 1,000/1. Taken together these data indicate that membrane surface viscosity dominates glycophorin diffusion in the reconstituted system and that the portions exposed in the aqueous phase have negligible effect. Assuming that the lipid viscosity in the erythrocyte membrane is the same order of magnitude as in DMPC, then the slow diffusion of band 3 proteins in erythrocytes must be attributed to protein-protein or protein-cytoskeleton interactions. The diffusion coefficients of reconstituted glycophorin in model membranes are consistent with Saffman and Delbruck's model.

M-PM-Po45 THE KINETIC MECHANISM OF ACTION OF THE WEAK ACID PROTONOPHORE FCCP. Roland Benz and Stuart McLaughlin. Fachbereich Biologie, Universität Konstanz, D-7750 Konstanz/ W.-Germany and Dept. Physiology and Biophysics, HSC, SUNY, Stony Brook, N.Y. 11794.

The kinetic mechanism by which weak acids such as FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone, pK ≈ 6) transport protons across bilayer membranes is difficult to determine because only very small current relaxations are observed on application of a voltage pulse. If the dielectric constant of the bilayer membrane is increased by using chlorodecane as a solvent, large relaxations in the current can be observed, which we studied using both the charge pulse and voltage clamp techniques. Upon application of a voltage clamp, the current, I(t), is described by an equation of the form $I(t) = I(0)\exp(-t/\tau) + I(\infty)$ where I(0) is the initial current, $I(\infty)$ is the steady state current and τ is the time constant. We measured I(0), τ and the amplitude of the relaxation, $\alpha = \{I(0) - I(\infty)\}/I(\infty)$, as a function of the pH, the concentration of FCCP and the applied voltage. At a given voltage and pH, I(0) increases linearly with the concentration of FCCP for [FCCP] < 1 μM. α increases with the pH. For example, when [FCCP] = 0.1 μM and V = 100 mV, $\alpha \approx 3$ at pH 7.3 whereas $\alpha \approx 30$ at pH 8.3. The rate constants were determined from an analysis of both the voltage clamp and charge pulse data. The rate limiting step is the back diffusion of the protonated form of FCCP. Similar results were obtained using CCCP. The kinetic mechanism of protonophore action observed with these chlorodecane-containing bilayer membranes may be of biological relevance; it has been suggested that the bilayer component of the mitochondrial membrane has a higher effective dielectric constant than a solvent-free or a decane-containing artificial bilayer membrane (Physiological Reviews, 60, 825, 1980). (Supported by NSF grant PCM 7903241.)

M-PM-Po46 INORGANIC MERCURY (Hg^{2+}) TRANSPORT THROUGH LIPID BILAYER MEMBRANES. John Gutknecht and Anne Walter, Physiology Dept., Duke Univ., and Duke Marine Lab., Beaufort, NC 28516

Diffusion of inorganic mercury (Hg^{2+}) through planar lipid bilayer membranes was studied as a function of chloride concentration and pH. Membranes were made from egg lecithin plus cholesterol (1:1 mol ratio) in tetradecane. Tracer (^{203}Hg) flux and conductance measurements were used to estimate the permeabilities to ionic and nonionic forms of Hg. At pH 7.0 and [Cl^-] ranging from 1-1000 mM, only the dichloride complex of mercury ($HgCl_2$) crosses the membrane at a significant rate. However, several other Hg complexes (HgOHCl, $HgCl_3^-$ and $HgCl_4^{2-}$) contribute to diffusion through the aqueous unstirred layer adjacent to the membrane. The relation between the total mercury flux (J_{Hg}), Hg concentrations and permeabilities is: $1/J_{Hg} = 1/P^{ul}\ [Hg^t] + 1/P^m\ [HgCl_2]$, where $[Hg^t]$ is the total concentration of all forms of Hg, P^{ul} is the unstirred layer permeability, and P^m is the membrane permeability to $HgCl_2$. By fitting this equation to the data we find that $P^{ul} = 1.5 \times 10^{-3}$ cm sec^{-1} and $P^m = 1.3 \times 10^{-2}$ cm sec^{-1}. At Cl^- concentrations ranging from 1-100 mM, diffusion of Hg^t through the unstirred layer is rate limiting. At Cl^- concentrations ranging from 500-1000 mM, the membrane permeability to $HgCl_2$ becomes rate limiting because $HgCl_2$ comprises only about 1% of the total Hg. Under all conditions, chemical reactions between Hg^{2+} and Cl^- near the membrane surface play an important role in the transport process. Other important metals, e.g., Zn^{2+}, Cd^{2+}, Pb^{2+}, Ag^+ and Sn^{2+}, form neutral dichloride complexes under physiological conditions. Thus, it is likely that chloride can "facilitate" the diffusion of a variety of metals through lipid bilayer and biological membranes. Supported by NIH grants ES 02289 and HL 12157.

M-PM-Po47 THE INTERACTION OF CATIONIC AMPHIPHILE WITH PHOSPHATIDYLGLYCEROL IN LIPOSOMES MADE FROM A MIXTURE OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLGLYCEROL. Howard H. Wang and Jeff M. Hall* Division of Natural Sciences, University of California, Santa Cruz, California, 95064

The ability of amphiphiles to bind to membranes by intercalating into the lipid bilayer is well established. However, little is known about the role of electrostatic charge in determining the interaction between charged amphiphiles and phospholipids in the membrane. We have attempted to estimate the proportion of cationic amphiphiles interacting with phosphatidylglycerol (PG) in bilayer liposomes made from a mixture of phosphatidylcholine (PC) and PG. We used a tertiary amine spin label 2-[N-methyl-N-(2,2,6,6-tetramethyl-piperidinoxy)] ethyl p-hexyloxybenzoate as the amphiphile. This amphiphile, which has a nitroxide reporter group located in the hydrophilic end of the molecule, is a particularly good probe for detecting mobility changes of the polar groups. (This probe, therefore, reports different molecular information than fatty acid spin labels which are sensitive to changes in the hydrocarbon region of the membrane.) The electron spin resonance (ESR) spectrum of this spin labeled amphiphile in bilayer liposomes composed of PC and PG is a composite of two spectra. The narrower of these two spectral components appeared to be identical to the ESR spectrum for pure PC liposomes in a similar experiment. Furthermore, using mixed PC-PG liposomes at pH 9.5 (where the spin labeled amphiphile is no longer cationic) the ESR spectrum appeared to be primarily composed of this narrower spectral component. These results suggest to us that the narrower spectral component arises from spin labeled amphiphiles which are not interacting electrostatically, while the broader component arises from spin-label amphiphiles bound to PG. Experiments were also carried out using liposomes of increasing PG to PC ratio; the results showed a proportionate increase of spin labeled amphiphile-PG interaction.

M-PM-Po48 THE ADSORPTION OF DIVALENT CATIONS TO BILAYER MEMBRANES CONTAINING NEGATIVE PHOSPHOLIPIDS. Alan McLaughlin, Arthur Lau, and Stuart McLaughlin. Dept. Biology, Brookhaven National Laboratory, Upton, N.Y. 11973 and Dept. Physiology & Biophysics, HSC, SUNY, Stony Brook, N.Y. 11794.

Zeta potential, calcium-sensitive electrode and ^{31}P NMR measurements were used to study the adsorption of divalent cations to bilayer membranes containing the negative lipids phosphatidylserine, PS, and phosphatidylglycerol, PG. All the results are consistent with the classical Gouy-Chapman-Stern theory of the diffuse double layer. The alkaline earth cations form mainly 1:1 complexes with lipids in unaggregated membranes and the intrinsic adsorption coefficients are all of the order of 10 M^{-1}. ^{31}P NMR measurements demonstrated that Ca has similar effects on the micropotential, the electrostatic potential at the binding site, and the macropotential, the average value of the surface potential calculated from the above theory. ^{31}P and ^{13}C NMR measurements demonstrated that Co forms inner sphere complexes only with the phosphodiester group of PG. About 1/3 of the adsorbed Co ions form inner sphere complexes with this group. A theoretical calculation indicates that the remaining fraction of adsorbed Co ions are not in the aqueous diffuse double layer; they are presumably adsorbed to the lipids in outer sphere complexes. Both the lack of selectivity among the alkaline earth cations and the magnitude of the intrinsic adsorption coefficients suggest that these cations also adsorb to phospholipids predominantly by forming outer sphere complexes. The relationship between the ability of divalent cations to adsorb to lipids and their ability to induce the aggregation of bilayer membranes will be discussed. (Supported by NIH grant GM24971, NSF grant PCM 7903241 and the USDOE.)

M-PM-Po49 THERMOMETRIC AND POTENTIOMETRIC TITRATION OF PHOSPHATIDYLSERINE VESICLES WITH DIVALENT CATIONS. S.J. Rehfeld[*a,c], N. Düzgüneş[b], D. Papahadjopoulos[b], and D.J. Eatough[*c], [a]V.A. Medical Center and [b]Cancer Research Institute, UCSF, San Francisco, CA 94143, and [c]Thermochemical Institute, BYU, Provo, UT 84602.

The interaction of divalent cations with small (∿25 nm diameter) unilamellar phosphatidylserine (PS) vesicles was studied by thermometric titration and potentiometric methods to understand the mechanism of aggregation and coalescence (membrane fusion) induced by these ions. The enthalpy associated with the addition of up to 0.5 mM Mg^{2+}, Ca^{2+}, Sr^{2+} or Ba^{2+} to PS vesicles (in 100 mM NaCl, pH 7.4) was <0.5 kcal/mol PS (endothermic) and is in agreement with values reported for the interaction of these ions with carboxylic acids. At higher concentrations an exothermic reaction was observed, whose magnitude was substantially greater for Ca^{2+} and Ba^{2+} than for Sr^{2+}. Mg^{2+} caused only a slight heat release. The binding isotherms of the divalent ions were determined using ion-selective electrodes. Potentiometric data on the complexing of Ca^{2+} with PS suggest that the end-point of the endothermic reaction was reached at 4:1 PS/Ca and that the threshold for the exothermic reaction coincided with 2:1 binding to PS in the outer monolayer of the vesicle. At the threshold [Ca^{2+}] the heat release was spontaneous and required no further addition of Ca^{2+}. Increasing the [Ca^{2+}] caused an additional exothermic reaction (∿2.5 kcal/mol). The reaction was complete at 1.6 Ca^{2+} added/total PS with a total heat release of 4.7 kcal/mol PS. Large (∿100 nm diameter) unilamellar vesicles gave a lower heat of reaction with Ca^{2+} compared to the small vesicles, and the reaction was completed at a higher concentration of the ion. The majority of the heat may be accounted for by the crystallization of the acyl chains of PS.

M-PM-Po50 EFFECT OF METAL IONS ON THE TRANSBILAYER DISTRIBUTION OF PHOSPHATIDYLGLYCEROL IN SMALL UNILAMELLAR VESICLES. B.R. Lentz, D.R. Alford* and C.S. Madden*. Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27514.

Oxidation with periodate followed by chromogenic determination of released formaldehyde has been used to distinguish phosphatidylglycerol (PG) exposed in the external leaflet of small unilamellar mixed PG/phosphatidylcholine (PC) vesicles from lipid located in the protected internal leaflet. Vesicles prepared in 100 mM NaCl, sodium cacodylate buffer (pH 7.2) displayed an excess of exposed PG over that expected for a random transbilayer distribution of lipid species. However, small amounts of Mn^{2+} added to the vesicle external space led to disappearance of the asymmetric distribution of both dipentadecyl/phosphatidylglycerol ($DC_{15}PG$; mixed with dimyristoyl phosphatidylcholine, DMPC) and egg-yolk-derived phosphatidylglycerol (mixed with egg phosphatidylcholine). Addition of EDTA at different times after Mn^{2+} additions gave assay results indicative of intermediate levels of asymmetry. In this way, we have determined the apparent first order characteristic times for Mn^{2+}-induced PG rearrangement to be 21 min at 110 phospholipid molecules per Mn^{2+} and 28 min at 10,000 phospholipid molecules per Mn^{2+} at a total lipid concentration of 0.11 mM in 1/1 $DC_{15}DG$/DMPC. Thin layer chromatographic analysis of the oxidized vesicles showed a single lipid species to be generated by oxidation and gave exposed to total PG ratios quantitatively in agreement with the chromagenic PG assay. Other ions were tested (Ca^{2+}, Cd^{2+}, Mg^{2+}, Pr^{3+}, Ti^{3+}, Eu^{3+}) but only Cd^{2+} and Mn^{2+} were found to exhibit this effect. The results have been interpreted to suggest that Mn^{2+} (and perhaps Cd^{2+}) catalyze the transbilayer redistribution of PG in small unilamellar vesicles. Supported by NSF (PCM-7922733) and USPHS (HL22771) and by an Established Investigator Award to BRL from the American Heart Association.

M-PM-Po51 ENCAPSULATION OF THE BIOINDICATOR OF Ca^{2+}, TnC_D, INTO LIPOSOMES. J.D. Johnson. Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Dansylaziridine Labeled Troponin C (TnC_D) has been shown to be a Ca^{2+} specific biological indicator of Ca^{2+} and rapid Ca^{2+} fluxes (Johnson et al. J. Biol. Chem. 253, 6451-6458, 1978, Ibid. 253, 5243-5246, 1978, Ibid. 254, 3497-3502, 1979). We have encapsulated TnC_{DANZ} into small unilamellar vesicles (SUVs) comprised of egg phosphatidyl choline, cholesterol and stearylamine, to form TnC_D-SUVs. Addition of Ca^{2+} to TnC_D-SUVs produces no change in TnC_D fluorescence. A 45% fluorescence enhancement is observed when TnC_D-SUVs in the presence of Ca^{2+} are perturbed by detergent lipases or Ca^{2+} ionophores. The pCa dependence of this fluorescence increase in TnC_D-SUVs in the presence of detergents or Ca^{2+} ionophores is identical to that of native TnC_{DANZ} indicating that incorporation of the labeled protein into liposomes does not affect its Ca^{2+} induced fluorescence changes. Any perturbation of lipid structure which permits entry of Ca^{2+} into the TnC_D-SUVs and the formation of the TnC_D-Ca^{2+} complex (i.e. ionophores, detergent or lipase action) may be accurately followed by the fluorescence increases in TnC_D. The fusion of TnC_D-SUVs (and delivery of TnC_D) to intact muscle will be discussed. This work was supported by grants from the NIH, HL 22619; Muscular Dystrophy Association and the American Heart Association (79-1001).

M-PM-Po52 EFFECT OF ANION SPECIES ON THE BINDING OF Ca^{++} AND Mg^{++} TO DIPALMITOYL-PHOSPHATIDYLCHOLINE BILAYERS. J.M. Collins, Selina Afzal*, Dong Sheng Guo*, John Hahn*, and L.J. Lis, Department of Physics, Illinois Institute of Technology, Chicago, Illinois 60616.

The net repulsive force as a function of the separation between various phosphatidylcholine bilayers has been previously measured in chloride solutions of different divalent cations (L.J. Lis, R.P. Rand and V.A. Parsegian (1980) Fed. Proc. 39: 1836). A non-linear Poisson-Boltzmann equation was used to convert this data to estimates of potential and charge density at the bilayer surface. It was found that the adsorbed charge decreased as the bilayers approached. We have examined the effect of anion species on this anomalous binding result to determine if the decrease in positive charge could be due to anion binding. By varying the anion species, we also expect to determine the effect of ionic size on the binding of anions to phosphatidylcholine. We will present preliminary results for dipalmitoylphosphatidylcholine bilayers in the presence of calcium and magnesium solutions of Cl^-, Acetate$^-$, NO_3^- and SO_4^{2-}.

M-PM-Po53 TRANSFER OF GLUCOCEREBROSIDE BETWEEN PHOSPHOLIPID VESICLES. M.C. Correa-Freire, Y. Barenholz, T.E. Thompson, Dept. of Biochemistry, University of Virginia, Charlottesville, VA, 22908 and Dept. of Biochemistry, Hadassah Medical School, Hebrew Univ., Jerusalem.

We have investigated the kinetics of transfer of glucocerebroside between phospholipid bilayers using 2-pyrenedecanoyl glucocerebroside (PyrCer) incorporated into dimyristoyl phosphatidylcholine (DMPC) vesicles as well as tritiated glucocerebroside incorporated into dipalmitoyl phosphatidylcholine (DPPC) bilayers as donor systems. Pyrene labelled cerebroside molecules are able to form an excited complex (eximer, E) between a PyrCer in the ground state and an excited momomer (M). When vesicles containing a known amount of PyrCer (donors) are incubated with unlabeled vesicles (acceptors), transfer of PyrCer from donor to acceptor populations will be reflected in a decrease of the observed E/M intensity ratio. The results obtained from these studies show that the half time of transfer at 37°C is >6.7 days. These results are confirmed using tritiated glucocerebroside incorporated into small unilamellar DPPC donor vesicles incubated with large unilamellar DPPC acceptor vesicles above the phase transition. Separation of the two vesicle populations by molecular seive chromatography at 45°C shows a half time of transfer greater than 30 days. We conclude that, contrary to the results found for phospholipids (Roseman, M. and Thompson, T.E. 1980. Biochemistry 19, 439), glucocerebroside does not readily transfer between bilayers under these conditions. This study was supported by U.S.P.H. grants GM-14628 and GM-23573.

M-PM-Po54 KINETICS OF TRANSFER OF GANGLIOSIDE FROM GANGLIOSIDE MICELLES TO PHOSPHOLIPID VESICLES. P.L. Felgner, R. Gillette, Y. Barenholz, R.P. Taylor, and T.E. Thompson, Biochemistry Department, University of Virginia and Hebrew University School of Medicine, Jerusalem, Israel.

A molecular sieve technique has been developed for the rapid separation of ganglioside micelles from 700 Å diameter unilamellar dipalmitoylphosphatidyl choline (DPPC) vesicles. This technique permits a determination of kinetics of transfer from micelles to vesicles. In the presence of excess ganglioside the level of incorporation into DPPC vesicles at 46°C saturates at about 12 mole % ganglioside. This saturation level is not markedly dependent on the number of sialic acid residues in the ganglioside. At ganglioside levels below saturation the rate of ganglioside incorporation into vesicles is independent of vesicle concentration. In addition the rate was found to depend on the sialic acid content of ganglioside with monosialo ganglioside being slow ($T_{1/2}$ 4 hrs.) and trisialo ganglioside fast ($T_{1/2}$ 0.5 hrs.). These results suggest that the transfer of ganglioside from micelles to vesicles is by diffusion of monomers through the aqueous phase and not by collision of vesicles and micelles. The data also suggest that the rate of transfer is limited by the rate at which monomers leave the ganglioside micelle. The net transfer of DPPC molecules to the ganglioside micelles is a very much slower process under these same conditions. This work was supported by USPHS grant GM14628 and GM23573 and by a US/Israel binational grant.

M-PM-Po55 PERMEABILITY OF SHORT-CHAIN FATTY ACIDS ACROSS BILAYER MEMBRANES: APPLICABILITY OF OVERTON'S RULE. Anne Walter and John Gutknecht. Department of Physiology, Duke University Medical Center, and Duke University Marine Laboratory, Beaufort, N.C.

The permeabilities of very small nonelectrolytes across biological and synthetic lipid bilayer membranes have been reported to be anomalously high relative to their hydrocarbon/water partition coefficients. In this study, a series of monocarboxylic acid permeabilities was measured for egg phosphatidylcholine-decane and phosphatidylethanolamine-squalane planar bilayers. 14-C-tracer fluxes were measured across membranes bathed with otherwise symmetrical well-buffered solutions at several pH's. Unstirred layer effects were taken into account by using high ratios of A^- to HA according to the relationship $1/J = 1/P^{ul}(HA+A^-) + 1/P^m(HA)$ where J is the total flux, P^{ul} and P^m the unstirred layer and membrane permeabilities, HA the combined and A^- the ionic form of the weak acid. P^m across egg PC-decane bilayers for formic, acetic, propionic, butyric and caproic acids were 110, 66, 260, 950 and 10,500 μm/sec, respectively. Formic and acetic acid permeabilities were higher than predicted by their alkane/water partition coefficients. The solubility diffusion hypothesis for nonelectrolyte permeation of lipid bilayers could explain these high permeabilities if the diffusion of the very small nonelectrolytes in the membrane is higher than predicted by the Stokes-Einstein equation relating the diffusion coefficient to viscosity. We tested this hypothesis by measuring the diffusion of water and the fatty acids in a series of hydrocarbon liquids with viscosities ranging from 3.3 to 672 cp and found that the diffusion of the small molecules is relatively insensitive to viscosity, i.e., the diffusion coefficient does not decrease as fast as the inverse of the viscosity. (Supported in part by NIH Grant HL12157).

M-PM-Po56 THE EFFECTS OF HYDRATION ON LATERAL DIFFUSION IN EGG LECITHIN MULTIBILAYERS. J.T. McCown, E.A. Evans, S.E. Diehl and H.C. Wiles. Duke University, Durham, N.C. 27706.

The fluorescence recovery after photobleaching technique (FRAP) was used to measure the lateral diffusion coefficient of the fluorescent lipid probe N-4-nitrobenz-2-oxa-1,3-diazole phosphatidylethanolamine (NBD-PE) in egg yolk phosphatidylcholine multibilayers as a function of water content. The degree of lipid hydration was controlled by equilibration with an atmosphere of known relative humditiy. As the relationships between relative humidity, weight fraction of water and area per lipid molecule are known, it is possible to correlate diffusivity with the area per lipid molecule. Over the range of relative humidities investigated (100% to 50%), an area per molecule decrease of approximately 20% is accompanied by a diffusivity reduction of nearly an order of magnitude. The diffusivity-area curve extrapolates to zero in the region of the L_α-L_β liquid crystalline-crystalline phase transition. These results demonstrate the strong dependence of lipid diffusivity on surface density. The lateral diffusivity decreases in approximately linear proportion to the reduction in free area per molecule produced by the removal of water. Diffusion coefficients are reported relative to that of a reference state of constant relative humidity. The use of relative diffusion coefficients allows changes in diffusivity to be accurately monitored without precise characterization of laser beam profiles. (Supported in part by USPHS NIH Grants HL23728 and HL16711.)

M-PM-Po57 INDUCTION OF ION PERMEABILITY IN LIPID BILAYER MEMBRANES BY THE FOOD DYE ERYTHROSINE B. Marco Colombini, Dept. of Zoology, Univ. of Maryland, College Park, MD 20742.

Food additives have been implicated in minimal brain dysfunction (including hyperactivity) in children. The underlying mechanism is not understood but an increase in transmitter release at the neuromuscular junction has been reported for the widely used food dye, erythrosine B. We tested the possibility that erythrosine B increased transmitter release by elevating cytoplasmic calcium levels by testing the ability of this dye to increase the permeability of lipid bilayer membranes to calcium. Erythrosine B does increase lipid bilayer permeability to ions including calcium, potassium and chloride. The required dosage of dye and the magnitude of the increased permeability to calcium are within ranges which allow the above hypothesis to be feasible. This permeability increase is dependent on the dye concentration raised to a high power (4 to 7 depending on dye concentration). This indicates the presence of permeability pathways consisting of as many as 7 monomers. Since erythrosine B is a small molecule (longest dimension is 15 A), a number of molecules would be needed if the bilayer were spanned. The presence of large permeability pathways which span the bilayer is indicated by observed fluctuations in the ion flux with time (dye dependent noise in the record). The permeability to ions induced by erythrosine B increases with increasing transmembrane voltage in a dramatic way indicating that it would be more effective on cells or organelles that have a large electrical potential difference across their membrane.

Supported by the General Research Board of the University of Maryland.

M-PM-Po58 SPIN-LABEL DETECTION OF ELECTROGENIC PROTON FLUXES IN PHOSPHOLIPID VESICLES.
David S. Cafiso and Wayne L. Hubbell, Dept. of Chemistry, Stanford Univ., Stanford, CA 94305 and Dept. of Chemistry, Univ. of California, Berkeley, CA 94720.

We have used a novel technique to characterize electrogenic proton fluxes in phospholipid vesicles formed by sonication or detergent dialysis. Using a number of spin-labeled hydrophobic ions, which allow a time-resolved determination of the transmembrane potential in small vesicle systems,* we have been able to monitor the electrogenic flux of protons in lipid vesicles across which a pH gradient has been established. Using a Nernst-Planck formalism, the time dependent proton flux has been used to generate an I-V (current-voltage) curve for the proton current in PC vesicles. The low effective monitoring impedance of our technique makes this possible. From the I-V curve, both the proton conductance and proton permeability have been estimated in PC vesicles. The proton permeability, P_{H+}, has been found to be strongly dependent upon the presence of small amounts of fatty acids or the common lipid solvent, chloroform. In vesicle preparations prepared as free from these contaminants as possible, we obtain proton permeabilities in the range of 10^{-8} to 10^{-9}cm/sec. I-V curves for the proton current are also being used to determine the work function for the transport of protons in these vesicle systems.

*D.S. Cafiso and W.L. Hubbell, Biochemistry, 17, 187 (1978).

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M-PM-Po59 EMISSION RESPONSE SPECTRA FOR CHARGE SHIFT PROBES OF MEMBRANE POTENTIAL
Logan L. Simpson, Leslie M. Loew, Alfred Hassner, and Rosario D'Costa, Department of Chemistry, State University of New York at Binghamton, Binghamton, N.Y. 13901

We have determined the potential dependent response of the emission spectrum for several p-aminostyrylpyridinium probes and some new more highly conjugated analogs. These response spectra are obtained from probes bound to a hemispherical lipid bilayer membrane. The excitation wavelength is set at the point where the transmittance response spectrum crosses zero, thus avoiding artifactual contributions from the potential dependence of the excitation spectrum. The emission responses can generally be interpreted in terms of an electrochromic mechanism as was the case for the transmittance or excitation spectral response described in earlier reports (Nature, 281, 497 (1979); Fed. Proc., 39, 2130 (1980)); some distortions in the emission response spectra from that ideally expected for electrochromism can be rationalized on the basis of geometric and orientational relaxation of the membrane bound excited state during its lifetime. Choice of optimal combinations of emission and excitation wavelengths can lead to fractional changes in fluorescence >5% for some of the newer probes.
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M-PM-Po60 CONDUCTANCE PROPERTIES OF LIPID BILAYERS MODIFIED BY PURIFIED FRACTIONS OF ALAMETHICIN.
Igor Vodyanoy†, James E. Hall†, T.M. Balasubramanian††and Garland R. Marshall†† (†University of California, Irvine, CA 92717;††Washington University, St. Louis, MO 63110).

The properties of two purified alamethicin fractions, fraction 4 and fraction 6, have been studied on phosphatidyl ethanolamine (PE) membranes and phosphatidylserine (PS) membranes. Membranes doped with fraction 4 show well-defined single channel conductance (mean lifetime about 20 msecs). The autocorrelation function of the conductance fluctuations has one relaxation time of the same order as the mean lifetime of the single channels, and the current response to a voltage pulse follows an exponential with only one time constant. The conductance of a membrane doped with fraction 6 has a voltage-independent part and a current-voltage curve with a slope half the slope of the fraction 4 current-voltage curve. In the presence of fraction 6, PS membranes and PE membranes both have symmetrical current-voltage curves even with fraction 6 added to only one side. We did not detect any well-defined single channel levels in the presence of fraction 6, and autocorrelation analysis of the conductance due to fraction 6 gave two characteristic correlation times: a fast time (5 msec) and a slow time (about 30 msec). High current level kinetics of fraction 6 also show two time constants. A possible explanation for the differences between the two fractions is that fraction 6 monomers have a lower dipole moment than those of fraction 4. The difference in channel stability is explained by the lowered tendency of the monomers to line up parallel to the field. The negative branch and voltage-independent conductance are explained by lowered energy of insertion of monomers into the membrane, and lowered energy of interaction between the monomers and the electric field. (Supported by HL 23183 and HL 00579 grants from the National Institutes of Health.)

M-PM-Po61 VOLTAGE INDUCTION OF TRANSIENT PORES IN UNILAMELLAR VESICLES OF DIPAMITOYL PHOSPHATIDYLCHOLINE Tian Yow Tsong and Justine Teissie, Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

A study of the voltage induction of transient pores in phospholipid bilayer vesicles is reported. Unilamellar vesicles with a size distribution of 100 nm were prepared by the method of Enoch and Strittmatter. The vesicles loaded with ^{14}C-sucrose, and suspended in a mixture of 150 mM NaCl and 272 mM sucrose, were exposed to an intense electric field in the range of 20 kV/cm to 40 kV/cm, with a field decay time of 5 μsec to 15 μsec. A transient leakage of sucrose label was detected when the field strength exceeded 30 kV/cm. After the field was removed no slow leakage of the tracer molecules occurred during a 65 hr incubation period at the room temperature (23 ± 2^{o}). The leakage is attributed to the field induced transmembrane potential, but not other effects such as the Joule heating or the shock wave associated with the voltage discharge. When this potential exceeded a threshold value of 200 mV, corresponding to an applied field strength of 30 kV/cm, there was a dielectric breakdown of the bilayer structure. Pores which allowed passage of sucrose were formed, transiently. Experiments showed that these pores were fully reversible, and no global and permanent damages to the vesicle bilayer were detected. The implication of this membrane potential-triggered conducting state of lipid bilayers to biological functions of cells or cell organelles will be discussed. This work was supported by NIH Grant HL 18048.

M-PM-Po63 INTERMEDIATE STEPS OF MEMBRANE FUSION. T. P. Stewart, L. T. Boni and S. W. Hui, Roswell Park Memorial Institute, Buffalo, New York 14263.

Fusion between phospholipid bilayers and between reconstituted membranes is induced by cations and freezing/thawing. Fusion between unilamellar vesicles, and between adjacent bilayers in multilamellar vesicles is observed by freeze fracture electron microscopy. Membrane samples are rapidly freeze quenched (without cryroprotectant) at various stages of fusion. The morphology of the membranes at each step is compared. In samples containing high percentages of unsaturated phosphatidylethanolamine, high curvature cusps (lipidic intramembrane particles) are seen to rivet adjacent bilayers together after fusion induced by freezing and thawing. These contacts are believed to be remnants of intermediate fusion sites. The so-called "lipidic particles" appear to be cusp-shape protrusion of bilayers rather than intramembrane "inverted micelles". Membrane disruptions during cation-induced fusion are also observed. Changes of molecular motion and packing are monitored by NMR and diffraction. A molecular model of fusion will be presented. Supported by NIH Grant GM 28120.

M-PM-Po64 PHOTOCHEMICAL STUDIES OF LATERAL DIFFUSION AND FUSION IN MODEL MEMBRANES. H. Williamson, C. G. Morgan, S. Fuller, and B. Hudson, Department of Chemistry, University of Oregon, Eugene, OR 97403.

Phospholipids containing one parinaric acid chain undergo a second order photochemical reaction in phospholipid bilayers. Two features of this reaction have been utilized in model membrane studies. One is that the rate of the reaction depends on the time for two molecules to diffuse to within a reaction radius. Hence a translational diffusion coefficient may be calculated from kinetic data as in Morgan et al. (PNAS, 77, 26 (1980)). We have determined the diffusion coefficient of parinaroyl labeled phospholipids in gel and fluid phase DMPC and DPPC injection vesicles containing cholesterol. In fluid lipids, the diffusion coefficient decreases slightly with increasing cholesterol concentration, in agreement with Rubenstein et al. (PNAS, 76, 15 (1979)). In gel phase lipids, an increase in the diffusion coefficient is seen around 30% cholesterol. The large increase reported by these authors at 20% cholesterol is not seen. The photochemical rate also depends on the local two demensional concentration of parinaroyl labeled phospholipids. We have used melittin, the principle lytic peptide of bee venom, to induce fusion of DPPC and EggPC injection vesicles. Parinaroyl labeled vesicles were mixed with unlabeled vesicles, and a decrease in photochemical rate, cuased by dilution of the label, indicated fusion had occurred. The lipid-melittin system had to be heated and cooled through the phase transition to fuse. Fusion was confirmed by electron microscopy. Contents are not retained during fusion of DPPC vesicles, as experiments with a soluble terbium complex indicate. EggPC vesicles either retain some contents or do not undergo complete fusion.

M-PM-Po65 HYBRID MEMBRANE FUSION. N. Düzgüneş, R. Straubinger*, and D. Papahadjopoulos. Cancer Research Institute, UCSF, San Francisco, CA 94143

The fusion of phospholipid membranes have hitherto been studied with systems in which all the liposomes in a suspension are of one particular phospholipid composition and of similar size. The development of a fluorescence assay for the intermixing of aqueous contents of the vesicles during fusion (Wilschut & Papahadjopoulos, Nature 281, 690, 1979) has enabled us to study the fusion of small unilamellar vesicles (SUV) with large unilamellar vesicles (LUV) of identical or different composition. Tb and dipicolinic acid (DPA) were encapsulated in the different vesicle populations; fusion resulted in the formation of a Tb/DPA complex and the enhancement in fluorescence. Small phosphatidylserine (PS) vesicles fused with LUV (PS) in the presence of >1 mM Ca^{2+}, a threshold concentration at which no fusion occurs between the large vesicles. Mg^{2+}, which induces limited fusion of small vesicles (Wilschut, Düzgüneş & Papahadjopoulos, Biochem., submitted), did not initiate any fusion between SUV and LUV at a broad range of divalent ion and lipid concentrations, indicating that both membranes must be highly curved for Mg^{2+} to induce fusion of PS membranes. Small PS/phosphatidylcholine (PC; 1:1) liposomes fuse preferentially with LUV (PS) and not with one another in the presence of 5 mM Ca^{2+}, analogous to their fusion with planar bilayers (Düzgüneş & Ohki, Biochim. Biophys. Acta, in press). At a threshold concentration of 1.5 mM, Ca^{2+} effected the fusion of SUV (PS) with large vesicles made of PS/phosphatidylethanolamine (PE; 1:1) and PS/PE/PC (5:4:1). The initial rate of fusion depended on the Ca^{2+} concentration and the SUV/LUV ratio. Extensive fusion could be induced by ≥2 mM Ca^{2+} between large PS and PS/PE (1:1 or 1:3) vesicles; Mg^{2+} was ineffective in this system although it did induce fusion among PS/PE LUV.

M-PM-Po66 TEMPERATURE DEPENDENCE OF CALCIUM-INDUCED FUSION OF VESICLES OF DIFFERENT PHOSPHATIDYLSERINES. S. O. Leung, S. K. Hark, E. P. Day and J. T. Ho. Department of Physics, State University of New York at Buffalo, Buffalo, New York 14260.

We have studied the calcium-induced fusion of sonicated bovine brain phosphatidylserine (PS), hydrogenated bovine brain phosphatidylserine (HPS) and dimyristoyl phosphatidylserine (DMPS) vesicles. Dynamic light scattering is used to measure the final vesicle size after calcium and EDTA incubation. The average final size serves as a measure of the extent of calcium-induced fusion of the sonicated vesicles. The extent of fusion as a function of incubation temperature shows a distinct maximum at 11°C for PS, 24°C for DMPS, and 38°C for HPS. Vesicles composed of mixtures of PS and HPS also exhibit a fusion peak temperature which increases progressively from 11°C to 38°C with increasing HPS concentration. The results are discussed in terms of a possible correlation between membrane fluidity and fusion.

Supported by NIH Grant GM-24590.

M-PM-Po67 REVERSIBILITY OF SODIUM-INDUCED AGGREGATION OF SONICATED PHOSPHATIDYLSERINE VESICLES. E.P. Day, J.T. Ho, A.Y.W. Kwok and S.K. Hark. Department of Physics, State University of New York at Buffalo, Amherst, New York 14260.

The kinetics of sodium-induced aggregation of sonicated phosphatidylserine vesicles has been studied as a function of sodium concentration and temperature. The concentration threshold for aggregation induced by monovalent sodium has been found to be 550 mM sodium by stopped-flow rapid-mixing techniques. This aggregation is completely reversible to changes in sodium ion concentration and to changes in temperature. The aggregation rate decreases with increasing temperature, indicating that the backward reaction rate increases more rapidly with temperature than does the forward rate.

Supported by NIH Grant GM-24590

M-PM-Po68 CALCIUM- AND MAGNESIUM-INDUCED FUSION OF MIXED PHOSPHATIDYLSERINE/PHOSPHATIDYLCHOLINE VESICLES. EFFECT OF ION BINDING. Nejat Düzgünes[1], Shlomo Nir[2], Jan Wilschut[1], Joe Bentz[2], Carolyn Newton[3], Archie Portis[4], and Demetrios Papahadjopoulos[1]. [1]Cancer Research Institute, UCSF, San Francisco, CA 94143; [2]Roswell Park Memorial Institute, Buffalo, N.Y. 14263; [3]Dept. of Biology, Kalamazoo College, Kalamazoo, MI 49007; [4]Dept. of Agronomy, U. of Ill., Urbana, IL 61801.

The aggregation, leakage and fusion of pure PS (phosphatidylserine) and mixed PS/PC (phosphatidylcholine) sonicated vesicles were studied by light scattering, the release of encapsulated carboxyfluorescein and a new fusion assay which monitors the mixing of the internal compartments of fusing vesicles. On a time scale of 1 min. the extent of fusion was considerably greater than leakage. The Ca^{2+} and Mg^{2+} concentrations required to induce fusion increased when the PS content of the vesicles was decreased, and/or when the NaCl concentration was increased. Calculations employing a modified Gouy-Chapman equation and experimentally determined intrinsic binding constants of Na^{+} and Ca^{2+} to PS, were shown to correctly predict the amount of Ca^{2+} bound in mixed PS/PC vesicles. For vesicles composed of either pure PS or of mixtures with PC in 100 mM NaCl (4:1 and 2:1 PS/PC), the induction of fusion (on a time scale of minutes) occurred when the amount of Ca^{2+} or Mg^{2+} bound/PS molecule exceeded 0.35-0.39. The induction of fusion for both pure PS and PS/PC mixed vesicles (with PS exceeding 50%) can be explained by assuming that destabilization of these vesicles requires a critical binding ratio of divalent cation to PS.

M-PM-Po69 INTERACTIONS BETWEEN MEMBRANES AS MEDIATED BY SPECIFIC LIGAND-RECEPTOR BINDING. Leaf Huang and Stephen R. Grant*. Dept. of Biochemistry, Univ. of Tennessee, Knoxville, 37916.

We have previously used the mono-octanoyl-α-bungarotoxin incorporated into liposomes to study the binding of liposomes with the microsac membrane vesicles isolated from the electroplaxes of Torpedo Californica as a model system to study the membrane-membrane interactions mediated by specific ligand-receptor binding. We have now prepared the mono-palmitoyl-α-bungarotoxin whose acyl moiety is twice as long as the octanoyl derivative and therefore provides a more stable anchor to the lipid bilayer. The purified mono-palmitoyl-α-bungarotoxin showed specific binding to the detergent-solublized acetylcholine receptors with a dissociation constant of 2×10^{-7}M, as compared to 1.16×10^{-8}M for the ^{125}I-α-bungarotoxin. Unilammellar liposomes with bound mono-palmitoyl-α-bungarotoxin showed specific binding to the microsac membrane vesicles but not to the red blood cell ghosts. Furthermore, the binding required a threshold ratio of toxin to lipid molecules in the liposome, suggesting a positive cooperativity for binding. We conclude that this system provides a simple model to study the interactions between membranes as mediated by specific ligand-receptor binding. Supported by NIH (CA24553) and Muscular Dystrophy Association.

M-PM-Po70 KINETICS OF SOLUBLE LIPID MONOMER DIFFUSION BETWEEN PHOSPHOLIPID VESICLES. J. Wylie Nichols and Richard E. Pagano, Carnegie Institution of Washington, Dept. of Embryology, 115 W. University Parkway, Baltimore, MD. 21210.

Two fluorescent methods have been used to study the kinetics of lipid transfer between small unilamellar vesicles. In the first, the transfer of the fluorescent phospholipid, 1-acyl-2-(N-4-nitrobenzo-2-oxa-1,3 diazole)-aminododecanoyl phosphatidylcholine, (C_{12}-NBD-PC) was monitored as it moved from donor vesicles containing self-quenching concentrations of the probe into unlabeled acceptor vesicles. In the second method, resonance energy transfer (RET) between 1-acyl-2-(N-4-nitrobenzo-2-oxa-1,3-diazole)-aminocaproyl, (C_6-NBD-PC) the energy donor and N-(lissamine) Rhodamine B sulfonyl dioleoylphosphatidylethanolamine (N-Rh-PE) the energy acceptor, was used to monitor the rate of C_6-NBD-PC transfer between the vesicle populations. Upon mixing donor vesicles containing both probes with nonfluorescent acceptor vesicles the rate of transfer of C_6-NBD-PC molecules between vesicle populations could be directly monitored.

Results from both of these techniques confirm that these probes are transferred between vesicles via the diffusion of free monomers and not by a vesicle collision-mediated process. A kinetic model is presented which predicts the rate of transfer and the equilibrium distribution of the soluble probes as a function of both the acceptor and donor vesicle concentrations and their respective on- and off-rate constants. By application of this model, we have shown that the rate at which an amphipathic lipid molecule enters or leaves a bilayer is dependent upon the structure of both its polar and non-polar regions as well as the composition of the vesicle bilayer. In addition, the half-time for equilibration as well as the equilibrium distribution of the phospholipid probes are dependent on its on- and off-rate constants which vary with the vesicle bilayer composition. (Supported by GM22942).

M-PM-Po71 FUSION OF DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES. M. Wong and T.E. Thompson, University of Virginia School of Medicine, Charlottesville, VA 22908.

Fusion of dipalmitoylphosphatidylcholine (DPPC) small sonicated vesicles (Schullery et al., (1980), Biochemistry V19, 3919) was studied as a function of temperature and vesicle concentration by Sepharose CL-2B chromatography and quasi-elastic light scattering. Fusion occurred faster at 4°C than at 21°C. At 4°C, increasing vesicle concentration increased the rate of fusion. At 4°C and 100 mM [Pi], small unilamellar vesicles fused entirely to ~700 Å diameter vesicles after 7 days, and these further fused to a population of larger diameter vesicles (~1000 Å) after 20 days. The ~1000 Å vesicles appeared homogeneous and unilamellar in freeze fracture electron micrographs. Studies at 4°C and 100 mM [Pi] found that small sonicated vesicles made from 50:50 co-lyophilized mixtures of DPPC and either (16:0/18:1)PC or (18:2/18:2)PC did not fuse appreciably over 2 days. 90:10 DPPC/(16:0/18:1)PC vesicles fused more slowly than pure DPPC vesicles, while 90:10 DPPC/(18:2/18:2)PC vesicles fused more rapidly. [Supported by USPH, NIH Grants GM-14628, GM-23573, and GM-07463.]

M-PM-Po72 LIPID-POLYETHYLENE GLYCOL INTERACTIONS. L. T. Boni, T. P. Stewart, J. L. Alderfer and S. W. Hui. Roswell Park Memorial Institute. Buffalo, New York 14263.

Fusion between small unilamellar vesicles of both egg phosphatidylcholine and bovine phosphatidylserine was induced by polyethylene glycol (M.W. 6,000), a known cell fusogen. Aggregation and fusion events were monitored by electron microscopy and turbidity measurements. The threshold concentration of polyethylene glycol for aggregation and fusion is found to be independent of lipid concentration. Typically, aggregation of phosphatidylcholine vesicles starts at 2.5% (w/w) polyethylene glycol but fusion is not significant until the polyethylene glycol concentration reaches 35%. Multilamellar vesicles were formed as a result of fusion. Polyethylene glycol is found to also promote the formation of structural defects in egg phosphatidylcholine, dimyristoyl phosphatidylcholine, and soybean phosphatidylethanolamine dispersions as shown by freeze fracture electron microscopy. ^{31}P NMR spectra of these dispersions reveal the existence of a non bilayer (isotropic) phase. The observed disruption in the bilayers may be associated with an intermediate stage of membrane fusion.

M-PM-Po73 SURFACE CHARGE AND GATING RATES OF THE K-CHANNEL OF THE SQUID AXON. J. F. Fohlmeister, W. J. Adelman, Lab of Biophysics, NIH Woods Hole, MA 02543.

In an extensive series of voltage clamp experiments whose protocol involved a series of calcium concentrations (2, 10, 40, 100mM) externally, each coupled with series of internal ionic strengths (863, 575, 288mM) in association with bulk potassium ion concentration gradients ($[K]_i/[K]_e$ = 450/5, 300/10, 300/50, 150/50, all mM) it was observed that the voltage shifts of the time constants for delayed rectification which normally accompany a $[Ca]_e$-series are a strong function of the K-gradients. These shifts are +6 to 9mV for an e-fold reduction of $[Ca]_e$ for 450/5 and 300/10; almost no shifts for 300/50; and -6 to -9mV/e-fold reduction of $[Ca]_e$ for 150/50. The time constants are independent of $[K]_i/[K]_e$ for bivalent cationic concentrations found in normal sea water. We conclude that electric field changes at the gating voltage sensor cannot be accounted for simply by the screening of fixed surface charge in conjunction with calcium binding. It seems likely that the local field due to conducting ions in preferential positions within closed channels is equally important.

The data were further evaluated in conjunction with the Grahame equation and the behavior of instanteous conductance to determine the distribution of fixed charge near the channel at the internal and external membrane surfaces. There appears to be a negligible amount of charge on the internal surface both near the "pore" opening and in a position to influence the voltage sensor for the channel gate. Further, there appears to be little change near the external "pore" opening, but charge with relatively strong calcium binding properties (association constant = $30M^{-1}$) in a position to influence the channel gating "machinery".

M-PM-Po74 ACETYLCHOLINE INHIBITION OF SLOW INWARD CURRENT IN VENTRICULAR MUSCLE. Ira Josephson and Nick Sperelakis. Univ. of Va., Physiol. Dept., Charlottesville, VA 22908.

In atrial muscle acetylcholine (ACh) decreases the slow inward current (I_{si}) and increases the time-independent outward K^+ current. However, in ventricular muscle ACh produces a marked negative inotropic effect only in the presence of certain positive inotropic agents which elevate intracellular levels of cyclic AMP. In this study, a two-microelectrode voltage clamp method was used on cultured reaggregates of cells obtained from 18-day-old embryonic chick ventricles to determine the effects of ACh on I_{si} and outward current during exposure to isoproterenol. Only double-penetrations displaying low-resistance coupling were selected for voltage clamp experiments. The cultured reaggregate preparation provides certain advantages over intact ventricles: (1) their small size (50-200 μm) facilitates control of membrane potential during I_{si} and facilitates adequate space clamp, and (2) the absence of autonomic nerve terminals permits examination of the direct effects of agents on membrane currents. Experiments were conducted in the presence of tetrodotoxin (10^{-6} M), and at a holding potential of -50 to -40 mV to eliminate the fast Na^+ current. Depolarizing voltage steps above -40 mV caused a slow inward current to flow which was sensitive to changes in $[Ca]_o$ and was blocked by verapamil (10^{-6} M). The maximal peak I_{si} occurred at -10 mV and it reversed around +30 mV. The addition of the β-adrenergic agonist isoproterenol (10^{-6} M) increased the magnitude of I_{si} at all clamp potentials. ACh (10^{-6} M) rapidly reduced the I_{si} to control values (before isoproterenol) without significant effects on either the instantaneous or delayed K^+ conductances. These effects were reversed by atropine (5×10^{-6} M). These results are consistant with a muscarinic inhibition of the adenylate cyclase leading to a decrease in cyclic AMP levels. We believe that a decrease in slow channel phosphorylation reduces their availability and, therefore, Ca^{++} influx during excitation.

M-PM-Po75 EFFECTS OF EXTERNAL RUBIDIUM ON POTASSIUM EFFLUX IN DEPOLARIZED FROG SKELETAL MUSCLE. B. Spalding, J. Swift, O. Senyk and P. Horowicz. Dept. of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642

$^{42}K^+$ efflux was measured in isolated sartorius muscles equilibrated in 305 mM K^+, 120 mM Cl^- with the membrane potential being held by chloride ions at -2 mV. K^+ efflux falls by a factor of 10 to 20 when Na^+ replaces all the external K^+. In the absence of external K^+, Rb^+ replacement of Na^+ produces an increase of K^+ efflux. As $[Rb^+]_o$ is raised from zero, K^+ efflux increases along an S-shaped curve and has a maximum at $[Rb^+]_o \simeq 30$ mM. As $[Rb^+]_o$ is further increased to a concentration of 300 mM the K^+ efflux gradually declines but is still higher in $[Rb^+]_o = 300$ mM than in Na^+ alone. K^+ efflux at $[Rb^+]_o = 30$ mM and $[Rb^+]_o = 300$ mM is about 1/3 and 1/5 respectively of that when $[K]_o = 305$ mM. As $[K^+]_o$ is raised from zero, K^+ efflux is increased along an S-shaped curve. When 10 mM external Rb^+ is added to K^+ containing solutions when $0<[K^+]<30$ mM there is a marked increase in K^+ efflux which is greater than that produced when 10 mM external Rb^+ is added when $[K^+]_o=0$. When $[K^+]\geq 45$ mM addition of 10 mM external Rb^+ produces a decrease in K^+ efflux. For $[K^+]_o \geq 75$ mM, the decrease in K^+ efflux produced by adding Rb^+ depends on the $[Rb^+]_o/[K^+]_o$ ratio (R). The fraction of K^+ efflux remaining after adding Rb^+ (r) is given by the relation $r = (1+\delta R)^{-1}$ where $\delta \simeq 2.0$. These results indicate that external Rb^+ has both an activating and a blocking action on the K^+ inward rectifier in muscle. The effects of Cs^+ are similar to those of Rb^+. For $[K^+]_o \geq 75$ mM, the decrease in K^+ efflux by Cs^+ differs only slightly from that by Rb^+. In the absence of external K^+, Cs^+ replacement of Na^+ ions has, however, only a very small activating effect. Supported by grants from the USPHS and MDA.

M-PM-Po76 THE ACTION OF BATRACHOTOXIN ON SODIUM CURRENT IN INTERNALLY-PERFUSED NEUROBLASTOMA CELLS. Li-Yen Mae Huang, Nava Moran, and Gerald Ehrenstein, NINCDS, NIH, Bethesda, MD 20205.

The ion flux method has frequently been used to study the interactions of neurotoxins and drugs with sodium channels in neuroblastoma cells. In order to appreciate the significance of results obtained by this method, it is necessary to know the properties of neuroblastoma sodium channels after they are modified by BTX.

We have studied the effect of BTX on sodium channels in cultured neuroblastoma hybrid cell line NG 108-15 under voltage clamp and internal perfusion. This was accomplished by using the suction pipette method. Our results show that after addition of BTX there is a gradual reduction of normal sodium current and a concurrent development of a new component of sodium current. Although this BTX-modified sodium current does not inactivate, it is otherwise qualitatively similar to normal sodium current. For the modified sodium channels, the activation region is shifted about 40 mV in the hyperpolarized direction and the rate of activation is substantially slowed. Thus, the effect of BTX on sodium channels in neuroblastoma cells is similar to its effect on sodium channels in frog myelinated nerve fibers.

M-PM-Po77 THE MONOVALENT CATION SELECTIVITY OF THE Ca^{2+} ACTIVATED K^+ CHANNEL. J.C. Woolum and A.L.F. Gorman, Dept. of Physiology, Boston Univ. School of Medicine, Boston, MA 02118

The calcium activated potassium current is found in a variety of excitable and non-excitable cells. The Ca^{2+} activated K^+ current of the molluscan neuron soma membrane was studied to determine whether the selectivity of the channel for various monovalent cations was similar to the delayed rectifying K^+ channel or to the leakage channel. The relative permeabilities of the Ca^{2+} activated K channel for different monovalent cations were measured using the reversal potential method described by B. Hille (J. Gen. Physiol., 1973, 61, 669). Cells in the abdominal ganglion of Aplysia were voltage clamped (two electrode voltage clamp)and a third electrode was used to inject Ca^{2+} ions by iontophoresis into the soma near the inner membrane surface. We find that the Ca^{2+} activated K^+ current is quite selective. The order for the selectivity of the channel relative to that for K^+ ions (P_X/P_K) is $P_K(1.0)>P_{Tl}(0.98)>P_{Rb}(0.7)>P_{NH4}(0.12)>P_{Cs}(0.03)>P_{Li}(>0.011)$, $P_{Na}(<0.009)$ and is similar to that found for the delayed rectifier K^+ channel of myelinated axon and of the internally perfused molluscan neuron soma (the Na^+ and Li^+ permeabilities are too small to measure perfectly with this technique). For the molluscan neuron Ca^{2+} activated K^+ channel the permeability ratio $P_{Cs}/P_K = 0.03$ whereas for the delayed rectifier K^+ channel of myelinated axon $P_{Cs}/P_K = 0.077$. Our results suggest that the mechanism responsible for channel selectivity (channel size, etc.) are similar for the Ca^{2+} activated and the delayed rectifier K^+ channels, but differ from those for the leakage channels. (Supported by NIH Grant NS 11429)

M-PM-Po78 THE KINETICS OF THE Ca^{2+} ACTIVATED K^+ CURRENT. J.C. Woolum and A.L.F. Gorman. Intr. by J. Head. Boston University School of Medicine, Boston, MA 02118

We studied the time course of activation of the Ca^{2+} activated K^+ current of the molluscan neuron soma membrane at different potentials to determine whether its kinetics are similar to those of voltage dependent K^+ currents or to those of leakage currents (or to neither). Cells in the abdominal ganglion of Aplysia were voltage clamped (two electrode voltage clamp) and a third electrode was used to inject Ca^{2+} ions by iontophoresis into the soma near the inner membrane surface. The membrane was depolarized (or hyperpolarized) briefly (<100 msec.) to various potentials from a holding potential near the K^+ equilibrium potential in a Ca^{2+} free external media containing 10 or 200mM K^+, and TTX. The current responses were digitized and the difference between currents before and after a 20 sec. Ca^{2+} injection was obtained and used as the Ca^{2+} activated K^+ current at each potential. Cells with and without axons were studied. The onset of the Ca^{2+} activated K^+ current started without a delay at all potentials, but the current changed during the pulse. The time to one half the maximum current (half time) depended upon membrane potential, i.e. the half time increased as the membrane was depolarized and decreased as it was hyperpolarized. There was no indication that the current inactivates (at least during potential steps of 100 msec. or less). The current at any potential did not follow a simple exponential time course, or a time course determined by two exponentials or by a single exponential raised to a power greater than 1. The kinetics of the Ca^{2+} activated K^+ current differ from voltage dependent K^+ currents which become faster rather than slower at potentials where the membrane K^+ conductance is increased. Our results show that activation of the Ca^{2+} activated K^+ current depends upon time as well as upon voltage and upon the intracellular Ca^{2+} concentration. (Supported by NIH grant NS 11429)

M-PM-Po79 VOLTAGE CLAMP KINETIC ANALYSIS OF ION CHANNEL KINETICS IN GIANT MUSCLE FIBERS OF BALANUS NUBILUS. H.B. Jachter, Dept. of Biophysical Science 118 Cary Hall, SUNY/Buffalo, Buffalo, N.Y. 14214.

Due to the complex membrane geometry of barnacle muscle fibers and the consequent lack of isopotentiality, it has been prohibitively difficult, in the past, to extract information about ionic channel kinetics. In order to obtain kinetic information I have rapidly washed out inhibitors from the fiber surface and monitored fiber currents during the course of washout. Using a current subtraction procedure I was able to quantify the kinetics of the isopotentially depolarized surface. Cobalt was used to inhibit inward calcium channel currents and TEA was used to block potassium currents. Where as calcium kinetics in other systems have been reported to be first order (1,2), preliminary results indicate that the inward calcium current in barnacle muscle turns on with sigmoidal kinetics. I have also observed that approximately half of the current from an unblocked fiber can be accounted for by currents passing through surface membranes alone. I have constructed a mathematical cable model to examine the ramifications of these channel distributions and kinetics by treating the cleft and tubular system as non-linear cables located in a myoplasm with ohmic series resistance. The time dependent Fourier coefficient variation of parameters method used appears to be more efficient than the Crank-Nickelson algorithm which has previously been applied to this problem. Supported by NSF PCM 76-81007 & NIH HL21294.

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M-PM-Po80 SODIUM CURRENTS IN SINGLE CARDIAC PURKINJE CELLS. F. Sachs & P. Specht*. Biophysics Dept., SUNY/Buffalo, NY 14214 & *Univ. Puerto Rico Sch. Med., San Juan, PR 00936.

Free running canine cardiac Purkinje strands were dissociated with collagenase and hyalauronidase. Electron microscopy revealed no basement membrane and no T system. Unfixed sarcomere length averaged 1.9 micra. Passive electrical properties of the cells were: 200-500pF; 2 (+-ISD) uF/cm^2; membrane resistance 1-5 kohm/uF; resting potential -20mV. The low resting potential was due to a TTX insensitive sodium leak, which could be revealed by choline substitution. With a fast microelectrode voltage clamp, sodium currents could be evoked from potentials negative to -80mV. These currents could be blocked by 10uM TTX and reversed by substitution of choline for sodium. Sodium inactivation occurred 20mV more negative than expected from the literature. The g-V curve had an initial slope of about 4mV/e and a midpoint of -40mV. INa peak was 1-2 mA/uF, with a maximum permeability, assuming constant field, of 2x10^{-4}cm/sec. Using m^3h kinetics, τ_h vs V was bell shaped with a peak of 40msec (-90mV, 16°C), and a Q10 of 10. τ_m at 16°C varied from 1msec(-51mV) to 0.2msec (+50mV) and had a Q10 of about 5. No divalent ion currents were visible. Addition of 20mM Ba caused a rightward shift in the Gna-V curve, a reduction in the maximum voltage sensitivity, and a profound blockage of the peak sodium conductance.
Supported by HL21294 and Heart Assoc. of WNY.

M-PM-Po81 ETHANOL DECREASES DISSOCIATION OF AGONIST FROM NICOTINIC CHANNELS. D.J. Nelson and F. Sachs. Intro. by V.S. Vaidhyanathan. Biophysics Dept., SUNY/Buffalo, N.Y. 14214

We have measured the effect of ethanol on suberyldicholine (SUB) activated single channel currents in tissue cultured chick skeletal muscle cells using the extracellular patch clamp technique(1). With ethanol in the pipette, over a concentration range of 1.5 to 5.0% (250 mM to 1.0 M), channel currents occurred in bursts. At low (5 nM) agonist concentrations, bursts were of uniform amplitude, suggesting that each burst represented the activity of an individual channel, rather than activation of independent channels. Burst length remained constant at approximately 22 msec and independent of ethanol concentration. The channel open time within a burst was essentially equal to that observed in the absence of alcohol (3.5 msec at 24 C), whereas the closed time decreased with ethanol concentration from 8 msec at 250 mM to 3 msec at 1 M. At 1 M ethanol, the time between bursts appeared to have two exponentially distributed components with time constants of 1.8 sec and 50 msec. Assuming the simplest sequential model of binding and opening, A+R↔AR↔AR*, where AR* is the conducting liganded state, and A is the agonist, the mean number of openings per burst is k_o/k_D(2), giving a dissociation constant for SUB at 1 M ethanol of 0.04/sec. The interburst times are most simply interpreted as the time required to bind one agonist and open (50 msec) and the time required to bind two agonists and open (1.8 sec). These opening rates are very close to diffusion limited rate at 5 nM of 0.3/channel/sec. Combining the dissociation rate with the apparent association rate gives an equilibrium constant of about 40 nM in the presence of 1 M ethanol. Supported by NS 13194 and 06271.
(1) Neher, E., Sakmann, B. and Steinbach, J.H. (1978), PFLUG ARCH. 375: 219-228.
(2) Colquhoun, D. and Hawkes, A.G. (1977). PROC. ROY. SOC. LOND. B. 199: 231-262.

M-PM-Po82 ARE THE LOCAL ANESTHETIC EFFECTS OF LIDOCAINE ENHANCED BY LIDOCAINE'S EFFECT ON POTASSIUM CONDUCTANCE? Gary A. Gintant and Brian F. Hoffman, Columbia University, New York, New York. 10032

Lidocaine is more effective in reducing the maximum rate of rise of the upstroke ($\dot{V}max$) of depolarized cardiac fibers than hyperpolarized cardiac fibers. Since the rate of depolarization reflects the sum of both outward and inward currents, and lidocaine may augment net outward potassium conductance as well as blocks fast inward sodium current, we questioned whether the augmented potassium conductance contributed to the reduction of $\dot{V}max$ observed at depolarized resting membrane potentials (RMP) observed with lidocaine. To study the relationship between RMP and $\dot{V}max$, we used standard microelectrode techniques to monitor transmembrane action potentials of canine cardiac Purkinje fibers as we varied RMP by varying $[K^+]_o$ during superfusion with lidocaine ($4X10^{-5}$ M.) and the potassium channel blocker CsCl ($5X10^{-3}$ M.). Superfusion with CsCl alone resulted in prolongation of the action potential duration and a decrease in membrane conductance, but did not effect the $\dot{V}max$ obtained at any RMP. Superfusion with lidocaine alone resulted in a slight reduction of $\dot{V}max$ at hyperpolarized RMP and marked reduction of $\dot{V}max$ at depolarized RMP. The reduction of $\dot{V}max$ by lidocaine was unaffected by the combined administration of lidocaine and CsCl. The reduction of $\dot{V}max$ by lidocaine is therefore independent of the effects of lidocaine on potassium conductance. These results also support the use of $\dot{V}max$ as a valid measure of fast inward sodium current.

M-PM-Po83 ESTIMATION OF KINETIC PARAMETERS OF ACETYLCHOLINE BINDING AT INTACT NEUROMUSCULAR JUNCTION, Bruce R. Land, Edwin E. Salpeter, Miriam M. Salpeter (Intro. by Roger Spanswick), Division of Biological Science and Dept. of Physics, Cornell University, Ithaca, NY 14853.

The relation between acetylcholine receptor (AChR) site density (σ) and the rising phase of the miniature endplate current was determined in esterase inactivated lizard intercostal neuromuscular junctions. The currents were recorded using a voltage clamp. The receptor site density was determined by electron microscope autoradiography after labeling with $^{125}I\alpha$-bungarotoxin in normal endplates and in those partially inactivated with non-radioactive α-bungarotoxin. We found that as the site density is decreased, the miniature endplate current rise time is increased and the amplitude is decreased. Furthermore, for any given value of σ, larger amplitude currents had longer rise times. From our data, we conclude that: (1) The most likely number of acetylcholine (ACh) molecules needed to open an ion channel is two. (2) The current rise time is due to σ-dependent factors such as diffusion and binding of ACh to AChR and to σ-independent time delays such as the conformation change time to open the ion channels. (3) The forward binding constant of ACh to AChR = $1.2^{+2.5}_{-.6}\ 10^8$ 1/MSec; the diffusion constant of ACh = $6.3^{+7}_{-2.5}\ 10^{-6}cm^2/sec$; and the channel conformational change time = 45 $\pm$ 15μSec (assuming a sequential, 2 step binding reaction coupled to ACh diffusion).

Supported by NIH grant NS09315 and MDA postdoctoral fellowship (BRL).

M-PM-Po84 APPLICATION OF THE BOLTZMANN TRANSPORT EQUATION TO ION MOVEMENT ACROSS BIOLOGICAL MEMBRANES. K.E. Cooper (Intr. by W.W. Sleator) Dept. of Physiology & Biophysics, U of Illinois, Urbana, Illinois 61801.

The exact mechanism of ion transport through ion-specific channels of biological membranes is not known. Two basic types of models have been put forth to explain this process. Historically, the first models were based on the Nernst-Planck electrodiffusion equation. The Nernst-Planck formalism has since been shown to be inadequate in a number of ways. Some researchers in the field have thus abandoned it and turned to Eyring rate theory models. Another approach is to inspect the foundations of the Nernst-Planck theory, see what assumptions in its derivation might be modified, and develop a theory of ion transport starting from a more fundamental kinetic equation. The more fundamental equation is the Boltzmann transport equation. An examination of this equation is undertaken to attempt the development of a kinetic formalism of more general validity than either the Nernst-Planck or Eyring formulations.

M-PM-Po85 EXPERIMENTAL EVIDENCE CONSISTENT WITH AGGREGATION KINETICS IN SODIUM CURRENTS IN MYXICOLA GIANT AXONS. G. Baumann, C.L. Schauf, and G.S. Easton (Intr. by J.J. Blum), Dept. of Physiol., Duke Univ. Med. Ctr., Durham, NC 27710, Dept. of Physiol., Rush Univ., Chicago, IL 60612, and Curr. of Oper. Res. and Syst. Anal., Univ. of North Carolina, Chapel Hill, NC 27514.

The Hodgkin-Huxley equations predict that if an axon during a maintained depolarization is subjected to a brief (50-200μsec) perturbing step of large amplitude (e.g. 200mV), the resulting perturbed current will always be larger in amplitude than the unperturbed current, and thus that the two records will never cross. In contrast, aggregation kinetics (Biophys. J. 25: 298a, 1979) predict that the perturbed current will cross over the response to a conventional pulse, and then remain smaller for the remainder of the depolarizing step. This has been experimentally tested using voltage-clamped Myxicola giant axons. Since we have shown that the presence of a large residual uncompensated series resistance can introduce an artificial crossing over, all experiments were performed in 10% Na^+ sea water, and adequacy of series resistance compensation tested by demonstrating invariance of the Na^+ kinetics as $[Na^+]_o$ was reduced. Under such conditions, crossing over still occurs, the magnitude of which varies systematically with membrane potential and time in a manner qualitatively consistent with that predicted from the aggregation model. A Markov characterization of a single hypothetical aggregation gating site allows for a molecular interpretation of these unusual kinetics. (Supported by NSF grant PCM78-02802 and NIH grant GM27260 to G.B., and NIH grant NS15741 to C.L.S.).

M-PM-Po86 MARKOV PROCESS CHARACTERIZATION OF A SINGLE AGGREGATION GATING SITE. Gilbert Baumann, Department of Physiology, Duke University Medical Center, Durham, NC 27710, and George S. Easton, Curriculum of Operations Research and Systems Analysis, University of North Carolina, Chapel Hill, NC 27514.

Channels responsible for excitability in membranes open and close at random. From this probabilistic nature of the single gating site the macroscopic theoretical kinetic behavior of a membrane containing many such sites is derived within the framework of the aggregation gating concept. Gating in the aggregation model involves a voltage-dependent reversible first-order reaction followed by a voltage-independent reversible aggregation process. The expected macroscopic behavior of a kinetic scheme involving few molecules can be analytically determined by a Markov formulation if the time intervals between individual events are assumed to be exponentially distributed. Using this method the basic macrokinetic properties of the potassium or sodium conductance system can be derived from an hypothetical aggregation gating site consisting of only 4 molecules. These results are quantitatively consitent with the average of a large number of individual sample paths generated by Monte Carlo simulations for the same parameters. Thus, the stochastic approach gives a complete and self-consistent description of some basic excitability phenomena that cannot be obtained from the conventional deterministic modeling approach. (Supported by NSF grant PCM78-02802 and NIH grant GM27260).

M-PM-Po87 UTILIZATION OF VOLTAGE-CLAMP DATA IN GENERATION OF CHANNEL-STATE MODELS. B.D. Fellmeth, and J.B. Nation*. Depts. of Physiology & Mathematics, Univ. of Hawaii, Hon., HI 96822.

Most attempts at modeling voltage clamp data, be it ionic conductance or gating current, begin with the assumption that the macromolecular gating machinery equilibrates between a finite number of thermodynamically distinct states, at least one of which conducts, with the interstate transition rates governed by first order rate constants, some of which are voltage dependent. If these assumptions are accepted, all artifact-free voltage clamp data can be expressed exactly as the sum of N exponential terms and a constant (C), where: N+1 is the total number of allowed states; the time constants of the exponentials are complex functions of the rate constants; C is the equilibrium occupancy and the pre-exponential factors are determined by the initial conditions. Although any model, based on the assumptions listed above, will also yield a conductance function of this form such functions are usually calculated numerically through "finite differences" integration of the rate equations (instead of using the inherent time constants in an exact exponential expression). Rather than matching data by trial and error adjustments of rate constants in empirically designed models, it may be advantageous to design models based on the properties and number of the exponential terms extracted directly from the voltage clamp data. Methods are presented for: a) extracting time constants and pre-exponential factors from raw data and, b) calculating these same parameters from the rate equations of state models. (Supported in part by BRSG Grant No. RR05599-13 and NIH Grant No. GM29263-01).

M-PM-Po88 EFFECTS OF SOLVENT SUBSTITUTION ON THE OCCLUSION OF NA^+ AND K^+ CHANNELS BY DRUGS AND IONS. C. L. Schauf, J. O. Bullock, and K. J. Smith*. Department of Physiology, Rush University, Chicago, Illinois 60612.

When substituted for normal water, deuterium oxide (D_2O) differentially slows various components of the Na^+ and K^+ channel gating processes, and reduces the maximum channel conductances. Nevertheless, there is no effect of D_2O on steady-state properties, intracationic selectivity, or intramembrane charge movements (Schauf and Bullock, Biophys. J. 29: 295, 2980). Sodium currents measured following early membrane repolarization ("tail" currents) are also insensitive to D_2O substitution. We have examined the effects of solvent substitution on the kinetics of occlusion of ionic channels by drugs (gallamine) and ions (Ba^{++} and nonyltriethylammonium). As might be expected from their presumed mechanisms of action, these processes are acutely sensitive to D_2O. For example, internal gallamine occludes Na^+ channels and the occluded channels do not inactivate normally so that large tail currents flow on repolarization. In contrast to normal tails, D_2O at 5°C slows the initial rate of gallamine occlusion by 40-50%, and slows the rate at which gallamine leaves the channel on repolarization by 70-80% (in axons in which initial activation was slowed by 35-40%). Internal barium (5 mM) produces a time- and voltage-dependent block of K^+ channels in Myxicola which is similar to that observed in squid axons (Armstrong and Taylor, Biophys. J. 30: 473, 1980). In this case the solvent-induced modifications are very complex, consisting not only of a slowing of the Ba^{++}-dependent K^+ inactivation (by amounts well in excess of the D_2O effects on K^+ activation), but also of changes in the steady-state Ba^{++} block. Such effects are additionally dependent on both membrane potential and external $[K^+]$. Similar effects are observed in the case of nonyltrimethylammonium. (Supported by USPHS grant NS15741 to C.L.S.)

M-PM-Po89 A COMPARISON OF SODIUM CHANNELS IN MYELINATED NERVE AND SKELETAL MUSCLE MEMBRANES: SENSITIVITY TO LOCAL ANESTHETICS. K.R. Courtney, Palo Alto Med.Res.Fdn., Palo Alto, CA

Sodium channels in skeletal muscle membrane are thought to be very similar to those in myelinated nerve. I have found that voltage-clamped skeletal muscle preparations [Bullfrog semitendinosis (ST) muscle] are much more sensitive to several local anesthetics. Comparisons of half-effective blocking doses (D_{50}) for both nerve and ST preparations appear in the table below. In addition, the rates of escape from channel block at hyperpolarized potentials [L(h=1)] is also listed in the table.

	mol.wt.	Nerve: D_{50}	L(h=1)	ST: D_{50}	L(h=1)
lidocaine	234	95 μM	~1 sec^{-1}	17 μM	1.7 sec^{-1}
tocainide	192	~300	~3	170	3.3
mexiletine	179	90	5.3	26	3.8

Nerve preparations with end-pools cut into CsF solutions (as ST preparations were) did not show enhanced sensitivity to mexiletine. The time constant governing development of inactivation (T_h) at depolarized levels was about 5 times faster in ST membranes that T_h values in nerve membranes at similar temperatures (10°C). T_h values at resting and hyperpolarized potential levels were only slightly faster in ST preparations. Despite observed differences in sodium channel blocking potency the relationship between drug size and speed of unblocking was similar to that seen in nerve with smaller drugs escaping from closed channels more rapidly. Supported by NIH grants HL 24156 and NS 15914.

M-PM-Po90 MODIFICATION OF SODIUM CONDUCTANCE KINETICS BY VENOM OF THE MARINE MOLLUSC CONUS STRIATUS. R. Hahin, G.K. Wang*, G.R. Strichartz, J. Schmidt*, and B.I. Shapiro. Depts. of Physiology & Biophysics and Biochemistry, SUNY, Stony Brook, N.Y. 11794 and NIGMS, Bethesda, Md. 20205

Venom of the piscivorous marine mollusc Conus striatus modifies the ionic channels in membranes of single myelinated axons of the frog. Exposure to the venom (15-30 minutes of 1.1 μg protein/ml) results in repetitive firing in response to stimuli which produce only single impulses in untreated fibers. Voltage clamp experiments revealed that Conus venom irreversibly affects both the sodium activation and inactivation processes. The maximum sodium permeability, P_{Na}, is reduced by about 50% and the voltage dependence of P_{Na} is shifted in the hyperpolarized direction by 16-20 mV. The rate of deactivation, measured by the decay of sodium current "tails" upon repolarization, is slowed 3-7 fold in venom-treated nerves. While shifts in the voltage-dependence of steady-state inactivation are small (6-9 mV negative) the rate of inactivation during a maintained depolarization (0 to -40 mV) is slowed by about 25%. Following an inactivating depolarization, the rate of reactivation during a period for which the potential was held at -100mV, was not significantly changed (one experiment). All these kinetic modifications are consistent with a stabilization of open sodium channels by Conus venom. Conus venom is a useful agent for studying both activation and inactivation.

M-PM-Po91 LOCAL ANESTHETICS NON-COMPETITIVELY INHIBIT BINDING OF TERBIUM, A FLUORESCENCE PROBE OF CALCIUM BINDING SITES, IN NERVE MEMBRANE VESICLES. R.J. Deschenes, D.C. Hilt, H.G. Mautner & J.K. Marquis (Intr. by R.E. Cathou), Dept. of Biochem. & Pharmacology, Tufts Univ. School of Med., Boston, MA 02111.

Terbium (Tb^{3+}) binds to membrane vesicles prepared from the walking leg nerve of the lobster (Homarus americanus) with a high affinity K_D of 2.2μM. Fluorescence of bound Tb^{3+} occurs via energy transfer from the aromatic residues of proteins(λ_{ex}=280nm; λ_{em}=546nm). Calcium inhibits Tb^{3+} binding competitively (linear K_{Dapp} vs. $[Ca^{2+}]$) with a K_i of 1.8 mM. Displacement studies with EDTA demonstrate that more than 95% of the bound Tb^{3+} is at the vesicle exterior and is not being taken up by the vesicles. To investigate the putative role of Ca^{2+} in the interaction of local anesthetics with axonal membranes, lidocaine and the analogues GX-HCl and QX-314 (Astra, Framingham, MA) were tested as inhibitors of Tb^{3+} binding. Inhibition by lidocaine is seen at considerably higher doses (25mM) than are required for conduction block of intact nerves (5mM). Inhibition by lidocaine and the primary amine analog GX-HCl is entirely non-competitive, whereas the quaternary ammonium derivative QX-314 appears to be a mixed competitive-non-competitive inhibitor of Tb^{3+} binding. These data are not compatible with the hypothesis that there is a functionally essential cation binding site on the axonal membrane surface for which Ca^{2+} and local anesthetics compete, although local anesthetic action may be modified indirectly by altered calcium concentrations. (Supported in part by NSF grant BNS-77-22356 and NIMH grant R3-MH-35155A.)

M-PM-Po92 EFFECT OF CALCIUM ON LOCAL ANESTHETIC INHIBITION OF AXONAL CONDUCTION IN LOBSTER (HOMARUS AMERICANUS) WALKING LEG NERVE BUNDLES. R.J. Deschenes and J.K. Marquis, Dept. of Biochemistry & Pharmacology, Tufts Univ. School of Medicine, Boston, MA 02111.

Although it has been shown (Blaustein & Goldman, J. Gen. Physiol. 49:1043, 1966) that Ca^{2+} can inhibit the conduction blocking action of local anesthetics in lobster peripheral nerve, it is not known whether Ca^{2+} directly competes for anionic membrane sites or indirectly alters the drug-membrane interaction. The present experiments were carried out over a sufficiently long time course to determine whether the blocking action of lidocaine is reduced by Ca^{2+} or whether Ca^{2+} only delays the onset of local anesthesia. 5mM lidocaine HCl was seen to reduce the amplitude of the compound action potential (AP) by 50% in 12 mins in normal lobster physiological saline (5mM $CaCl_2$), while in high Ca^{2+}(75mM)-saline, 5mM lidocaine reduced the AP amplitude by 50% only after 25 mins of external bath perfusion. The data confirm the suggestion by Strichartz (Anesthesiology 45:421, 1976) that Ca^{2+} might hinder the passage of local anesthetics across glial membranes. (Supported in part by NSF grant BNS-77-22356.)

M-PM-Po93 TETANIC HYPERPOLARIZATION BY SODIUM LOADING OF A SYSTEM IN WHICH ACTIVE TRANSPORT IS LINKED TO FRANKENHAEUSER-HUXLEY KINETICS. G.M. Schoepfle, J.T. Tarvin* and Richard M. Martin*. Dept. of Psychiatry, Neurosciences Program, Department of Physics and Dept. of Neurology, University of Alabama in Birmingham, Birmingham, Alabama 35294.

A displacement of the potassium activation curve together with an adjustment in P_K permits setting of a 3:2 ratio in the resting state for sodium vs potassium leak current densities as described by the Frankenhaeuser-Huxley system of excitation equations applicable to the Xenopus node. Non specific leak conductance g_L is set at .0303mho/cm^2. (Frankenhaeuser and Huxley, J. Physiol., 171:302, 1964). Net leak current density at rest is balanced by an active transport term I_p such that

$$I_p = g_p V - 3g_p\,(RT/F)\ln[(Na)_o/(Na)_i] + 2g_p(RT/F)\ln[(K)_o/(K)_i] - g_p E_0 \quad \text{where}$$

$$E_0 = g_p(RT/F)\ln[(ADP)(P_i)/(ATP)(H_2O)] + g_p\Delta H/F + g_p(T/F)[S^o_{ATP} + S^o_{H_2O} - S^o_{ADP} - S^o_{P_i}]$$

(Rapoport, Biophys. J. 10:246, 1970; Schoepfle and Tarvin, The Physiologist 22:113, 1979 and 23:111, 1980). E_0 is then uniquely determined by selection of g_p. Analysis of subsequent repetitive activity involves iterative numerical solutions of the differential equations which indicate that a quasi-steady state is quickly attained, with the result that successive voltage-time patterns appear identical. The initial value of the time dependent $(Na)_i$ is now adjusted to a level such that throughout the interspike interval the time integral of total sodium current vanishes. This condition uniquely determines the tetanic voltage time pattern corresponding to any given g_p. Choice of g_p is then dictated by extent to which tetanization alters the configuration of a control spike with respect to height and extent of post spike hyperpolarization. NIH support.

M-PM-Po94 GALLAMINE TRIETHIODIDE: TEA$^+$- AND PANCURONIUM-LIKE EFFECTS ON NERVE FIBERS. K. J. Smith* and C. L. Schauf. Department of Physiology, Rush University, Chicago, Illinois 60612.

Gallamine triethiodide ("Flaxedil") is a widely used neuromuscular blocking agent in clinical and experimental science. Voltage - clamp studies demonstrate that gallamine directly affects nerve fibers. External gallamine blocks the potassium conductance in frog myelinated nerve with an apparent dissociation constant of 10^{-4} M. At rat nodes of Ranvier, which normally lack a potassium conductance, gallamine blocks the g_K which can be induced by physical and chemical manipulations. The potassium conductance in Myxicola axons is unaffected by both internal and external gallamine. External gallamine does not affect Na^+ conductance in either myelinated nerve or Myxicola. The effects of internal application of gallamine to myelinated nerve and Myxicola resemble those produced by pancuronium in squid axons. Thus, internal gallamine slows Na^+ inactivation for small depolarizations; induces a two-component decline in I_{Na} (corresponding to a rapid occlusion of some Na^+ channels by drug molecules, and slowed inactivation) for moderate steps; and almost completely blocks outward Na^+ currents at potentials more positive than E_{Na}. In rat fibers and Myxicola, gallamine occluded channels fail to inactivate completely and large, slow inward Na^+ tail currents are observed as gallamine leaves occluded channels. In myelinated nerve, internal gallamine is a less potent inhibitor of g_K than external gallamine. Asymmetry current 'ON' responses develop a slow component in the presence of gallamine, while immobilization following long pulses is reduced or eliminated. (Supported by USPHS grant NS15741 and National Multiple Sclerosis Society grant 1313A2 to C.L.S, and by a NMSS fellowship to K.J.S.).

M-PM-Po95 HOW VALPROATE AFFECTS THE ACTION POTENTIAL,IONIC CONDUCTANCES,AND ACTIVE TRANSPORT CHARACTERISTICS OF THE CRAYFISH GIANT AXON. T. M. Nosek and M. Brasher - Crosland (Intr. by Edward M. Lieberman) Dept. of Physiology, Medical College of GA., Augusta, GA. 30912.

This series of experiments was undertaken to determine the effects of valproate (VPA), a new and very effective antiepileptic agent, on the electrophysiologic properties of the crayfish medial giant axon. The axons were space and current clamped in order to measure steady-state conductance (g_m). Extracellular stimulation generated conducted action potentials for analysis. 4mM VPA was the highest non-toxic dose. Within 30 min. it produced a few mV depolarization of the cell that was associated with a decrease in g_m. VPA decreased the magnitude of the action potential (AP), $+dV/dt_M$, $-dV/dt_M$, and increased the duration of the action potential (AP-D) suggesting that it affects both the voltage dependent Na^+ and K^+ channels. Exposure of the axon to a medium containing zero chloride (isethionate subsitution) produced no change in resting potential but significantly decreased g_m, $+dV/dt_M$ and increased AP-D. The Cl^- free medium did not significantly affect the response of the axons to VPA suggesting that VPA does not influence Cl^- conductance either at rest or during activity. Inhibition of electrogenic Na^+-K^+ active transport by ouabain also did not influence the membrane response to VPA. By using the depolarization in response to ouabain to calculate the net electrogenic active transport current, we used the analysis described by Lieberman and Nosek (Pflugers Arch. 366: 195, 1976) that is based on the steady-state equation of Hodgkin and Huxley to calculate the resting Na^+ and K^+ conductances and Na^+ -K^+ transport coupling ratio in control axons and axons pretreated with VPA. VPA was shown to be without effect on total pump current, pump coupling ratio, and g_{Na}. VPA did, however, decrease g_K. This accounts for the depolarization observed in response to VPA. (Supported by NIH #1-NS-6-2340 to the Georgia Comprehensive Epilepsy Program).

M-PM-Po96 MEASUREMENTS OF CALCIUM BUFFERING IN A NEURONAL CELL BODY. D. Tillotson & A.L.F. Gorman. Intr. by W. Ullrick. Boston University School of Medicine, Boston, MA 02118

Given the importance of accumulated Ca^{++} following excitation in a variety of cellular processes, it follows that the mechanism(s) responsible for Ca^{++} uptake and/or extrusion have significant, albeit indirect effects on the Ca^{++} dependent processes. We have studied cytoplasmic Ca^{++} removal using the absorbance change signal from single Aplysia neurons injected with Arsenazo III. Absorbance measurements were made during and following membrane excitation under voltage clamp and iontophoretic injections of Ca^{++}. In addition to studying the properties of Ca^{++} buffering, attempts were made to select from among the possible mechanisms those most important in buffering transient Ca^{++} accumulation following excitation of Ca^{++} channels. This was done by manipulations of bath temperature, removal of extracellular Na^+, application of reportedly selective inhibitors of various cellular machinery. These inhibitors included mitochondrial blockers (cyanide derivitives and ruthenium red), a possible endoplasmic reticulum blocker (caffeine), and a possible Ca^{++}-pump blocker (vanadate). While we found a significant slowing of absorbance signals with decreasing temperature measured with both Ca^{++} entry and Ca^{++} injection, no such similarity was observed when the pharmacological agents were applied. For example, it was possible to produce slowing of the measured absorbance change with injected Ca^{++} using the cyanide derivatives without producing an effect on the absorbance measurement associated with voltage clamp pulses. The evidence indicates that neither mitochondria nor a Na^+-Ca^{++} exchange systems play a significant role in the Ca^{++} removal mechanism seen following membrane excitation and are suggestive of the absence of a role played by endoplastic reticulum. The mechanism which is responsible for the short term Ca^{++} buffering remains unclear. (SUPPORTED BY NIH. NS11429)

M-PM-Po97 Calcium DEPENDENT INTRACEREBRAL BIOELECTRIC PROCESSES
Clara Torda- N.Y.Center for Psychoanalytic Training, New York,N.Y.,10028 (Research Dpt.).

Experimental observations have been collected on various intracerebral bioelectric processes related to the functions of the nervous system. Experimental data will be presented on calcium dependence of the hyperpolarization of the outer membrane of the photoreceptors of the retina of bullfrog, dependence on Calcium gating processes of encoding of sensory stimuli, the graded slow potentials (the encoders) and Ca-dependence of dendritic spike generation (the transporters). These processes will be documented on the granule cells of the dentate fascia and the pyramidal neurons of the hippocampus (CA3, CA1).The dependence on Ca-gating and Ca-binding processes of attenuation will be documented on the gigantocellular neurons of the pons of cat. Memory storage capacity of Ca-binding proteins will be documented on hippocampal slices of cat during behavioral events. The use of Ca during executory activity of control systems generated by serotonin will be documented on two behavioral process, also on some aspects of the sleep cycle. The mechanism of action of one of the acetylcholine receptor system involved in generating Ca transport has been identified. Several of the allocated processes have selfinitiating and selfregulatory capabilities.

M-PM-Po98 FLUORESCENT PROBE STUDY OF SYNAPTOSOMAL PLASMA MEMBRANE (SPM) DERIVED FROM ETHANOL-DEPENDENT RATS. Harish C. Pant, Charles E. Swenberg, Edward Majchrowicz* and Forrest F. Weight*. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism. Rockville, MD 20852.

Changes in Ca^{++} binding and membrane surface potentials of the SPM were explored by measuring the fluorescence in the presence and absence of added ethanol (10-100 mM). The hydrophobic fluorescence probe, 1-anilino-8-naphthalene sulphonate (ANS, 10 μM), and the calcium chelator probe chlorotetracycline (CTC, 50 μM) were used in saline solution (10 mM, pH, 7.26). The SPM were prepared from several regions of three groups of Sprague-Dawley rats (230-300g): water treated controls, dependent while still intoxicated, and rats undergoing overt ethanol withdrawal syndrome. Physical dependence was induced by peroral administration of ethanol (20% w/v) (Psychopharmacologia, 1975). The ASN fluorescence intensity increased 10 to 15% upon addition of exogenous ethanol to SPM isolated from cortex, hippocampus, hypothalamus and cerebellum from control animals. However, no changes were noted in SPM derived from colliculi. The SPM from intoxicated ethanol-dependent rats (blood ethanol concentrations 80-100 mM) showed no significant changes in the ASN fluorescence with the addition of ethanol. The changes in the fluorescence of SPM derived from rats undergoing overt withdrawal syndrome (no blood ethanol present) varied with the region of brain and were either similar to those from the controls or those from dependent intoxicated rats. A similar pattern of responses was noted in SPM with chlorotetracycline added, although the difference in responses between controls and ethanol treated rats were more pronounced. The results suggest that the SPM have undergone physical alteration upon induction of physical dependence on ethanol. This may be due to a change of ion binding or surface charge.

M-PM-Po99 GENERAL THEORY OF REVOLUTION (DISCONTINUOUS EVOLUTION)
Peter Fong, Emory University, Atlanta, Georgia 30322

The Darwinian theory of evolution is based on the accumulation of infinitesimal changes and so can explain continuous evolution (e.g., from limbs to fins), which is slow, but not discontinuous evolution (e.g., from quadruped to biped), which characterizes the evolution of man. The revolutionary changes can be explained by the principles of juxtaposition, transfiguration, etc., as formulated in an earlier paper,[1] and the pace of evolution can be rapidly accelerated by discontinuous jumps. Whereas the Darwinian mechanism leads to perfection (e.g., the streamlined body of whale), the juxtaposition of two systems, such as the solitary individual and the cooperative society, for the advantage of survival, may lead to intrinsic conflicts that can never be completely resolved. In fact, imperfection is the hallmark of man--from squabbles in the family to intrigues in the court. These were attributed to the original sin in Catholicism or dialectic contradiction in Marxism. Now they are taken out of the religious and ideological arenas and studied on a scientific basis. In the process such notions as love, compassion, glory and guilt are studied in the evolutional perspective and many moral and social issues may be analysed scientifically. Besides the biological and social evolution of man, the cultural evolution may also be studied on the same basis. There are inventions resulting from continuous evolution induced by the environment a la Darwin or Marx (pictorial writings, empirical sciences, despotic institutions). There are also more important inventions resulting from discontinuous changes through juxtaposition and transfiguration (phonetic writing, Newtonian science, democratic institutions). The ultimate goal of this study is to explain all social sciences and the humanities in terms of the second principles pertaining to biology that have been formulated on the first principles of physics.[1]

[1]P. Fong, in Biogenesis Evolution Homeostasis, A. Locker, Ed.,(Springer-Verlag, 1973),93-106.

M-PM-Po100 THE RATE OF TRANSITION AMONG THE MULTIPLE STABLE STEADY STATES IN A CHEMICAL REACTION SYSTEM. V. T. Kurtz. Department of Biophysical Sciences, SUNY at Buffalo, 118 Cary Hall, Buffalo, New York 14214.

In a chemical reaction system which can exhibit multiple steady states far from equilibrium, the stochastic nature of the reactions gives rise to transitions among the stable steady states. Such processes are not accounted for by the deterministic laws of mass action, which describe only the decays of the system to the stable steady states. A stochastic model, in which the reactions are considered to be Markov processes in a discrete state space (the possible amounts of each chemical species in the system) and in continuous time, can describe not only the relaxations to but also the transitions among the stable steady states. In order to examine such a process more concretely, a model reaction scheme which can exhibit three steady states was used. It was found that in the stochastic description of this reaction scheme, the transitions between the two stable steady states are accounted for by an exponential decay with a single time constant. A variation of the system parameters from a region with one steady state through a region with three steady states to another region with a single steady state was helpful in demonstrating the association of this time constant with the fluctuations between the stable steady states. The dependence of this time constant upon the size of the system is consistent with this interpretation.

M-PM-Po101 ELEVATED HYDROGEN PEROXIDE PRODUCTION BY MITOCHONDRIA ISOLATED FROM PULMONARY MACROPHAGES OF NEONATAL ANIMALS. Marga Herweijer and Anne P. Autor, Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

Pulmonary macrophages (PMs) obtained from neonatal rats differ biochemically from PMs of adult rats. It has been observed in this laboratory that neonatal rat PMs are very resistant to the toxic effects of oxygen radicals in comparison with adult cells. This resistance appears to be related to the 4 to 5 fold higher catalase and mitochondrial superoxide dismutase (SOD) content in neonatal rat PMs. Furthermore, mitochondria of neonatal PMs produce H_2O_2 at a greater rate when measured in the presence of succinate. The difference in rate is greatly magnified when H_2O_2 generation is measured in oxygen. Most available evidence indicates that O_2^- generated from one or more components on the electron transport chain, is the source of released H_2O_2 from the whole mitochondria. Washed sub-mitochondrial particles prepared from neonatal cells have an elevated rate of O_2^- production which decreases as the animal matures. In order to determine the source of the O_2^- production in neonatal cells, spectral analysis of the components of the electron transport chain was undertaken using mitochondria prepared from PMs of rabbits. Cells from these animals react similarly to rats, and can be obtained in higher quantity from fewer animals. Spectral analysis was conducted at -196°C in order to enhance the sensitivity. Differences in the spectra between neonatal and adult preparation were proposed to explain the enhanced H_2O_2 from neonatal cells. Other lines of evidence suggest that one or more of the components may be destabilized and, therefore, more easily autoxidized, thus giving rise to O_2^- and H_2O_2 at a greater rate than with the mature cell. It would be expected, as is the case, that more mitochondrial SOD would be present in the matrix to remove catalytically the O_2^- formed. (Supported by NIH GM 12675.)

M-PM-Po102 PERMEABILITY AND INTRACHANNEL CHARGE DENSITY IN A K^+ SELECTIVE CHOLINERGIC CHANNEL IN APLYSIA CALIFORNICA. T. L. Schwartz, BSG, Univ. of Conn., Storrs, CT 06268.

Membrane permeability to ions has been demonstrated to be a specified function of the electrical potential in the interior of the channels through which the ions pass (Schwartz, T.L. and Kado, R.T., 1977 Biophys. J. 18:323-349). But the relationship between intrachannel charge density and potential is specified by Poisson's equation. Thus permeability provides an index to intrachannel charge density. This has been explored in this channel. The observed permeabilities suggest a system in which each charged intrachannel site is associated with a counter-ion but in which a net bound charge results from polarization effects produced by the membrane potential itself acting on a channel system that is inhomogenous in that its dielectric constant varies as the channel is traversed. At a membrane potential of -100 mV @ 15°C and an external potassium concentration of 10 mM the intrachannel charge density is shown to be 150 $(10)^{-9}$ moles of positive univalent charges/cc of channel. At the same membrane potential, but an external potassium concentration of 20 mM, this charge density increased to 200 $(10)^{-9}$ moles/cc of channel. If the channels are assumed to be shaped like a 4Å pore--like the pores for water in erythrocytes, and the membrane is taken to be 70Å thick, at 10 mM external K^+ a single channel would contain 3.18 $(10)^{-4}$ univalent charges. At 20 mM external K^+ this increases to 4.24 $(10)^{-4}$ charges. Any of the currently reasonable models for channel shape yield similar low values of charge per channel. Indeed the relationship between intrachannel charge density and intramembrane electrical potential is almost linear. The slope of this curve at 20 mM external K^+ is steeper than that at 10 mM revealing a more polarizable channel at the higher external K^+. This explains the permeability's dependence on external K^+.