M-PM-K1 DIFFERENT STEPS IN THE REGULATION BY INTRACELLULAR $|Ca^{++}|$ AND pH OF THE ADH INDUCED HYDROSMOTIC RESPONSE. M. Parisi and J. Bourguet. Centre d'Etudes Nucléaires de Saclay, Département de Biologie, 91191 Gif sur Yvette Cedex, France.

The hydrosmotic responses induced by ADH (Oxytocin, 2.2 x 10^{-8}M) or 8-Br-cyclic AMP (10^{-3}M) in frog (Rana esculenta) urinary bladders were at minute intervals recorded. Once the response was developed, the Ca ionophore A23187 (10^{-5}M) or the Ca chelator EDTA (1 mM) were added to the mucosal or serosal baths (containing 1 mM $CaCl_2$), either at 22 or 12°C. Their effects were compared to the inhibition induced by CO_2 bubbling, in similar conditions. It was observed that 1) The response to oxytocin was reduced both by mucosal (Mean diff: 49±3%,n=12) or serosal (38±4%,n=11) ionophore addition. 2) The response to 8-Br-cyclic AMP was inhibited from the mucosal (38±4%,n=11) but not from the serosal (9±5%,n=6) side and 3) The half time of the ionophore action (5.1±03 min at 22°C) was strongly temperature dependent and similar to the oxytocin wash out. This was at variance with previous results observed when CO_2 bubbling was tested. 4) Mucosal EDTA inhibited (36±4%,n=9) the oxytocin response but the half-time of its action (1.9±0.3 min) was similar to the one observed during CO_2 inhibition. It can be concluded that 1) The Ca^{++} ionophore had access to different Ca^{++} pools when added to the mucosal or serosal solutions. 2) Mucosal addition of the Ca ionophore interfered with a temperature dependent post cyclic AMP step. 3) A reduction in the mucosal $|Ca^{++}|$ partially impaired the water channel. From these results can be interpretated that intracellular $|Ca^{++}|$ modulates the fusion of the water channel-containing vesicles with the apical membrane but, contrary to the case of intracellular low pH, did not modify the channel permeability state. Conversely, the reduction of mucosal $|Ca^{++}|$ had similar effects that an increase in intracellular pH.

M-PM-K2 BASOLATERAL KCl SYMPORT CONTRIBUTES TO TRANSCELLULAR Cl^- TRANSPORT IN NECTURUS GALLBLADDER. Ayus Corcia and William McD. Armstrong, Dept. of Physiology, Indiana University School of Medicine, Indianapolis, IN 46223

Cl^- enters Necturus gallbladder cells by a coupled NaCl transport mechanism located in the mucosal membrane of the epithelial cells. Passive Cl^- permeability across the basolateral membrane is much too low to account for the rate of transcellular Cl^- transport. A KCl symport mechanism in the basolateral membrane has been postulated to allow for Cl^- exit from the cells. The driving force for this mechanism would be the chemical potential gradient for K^+ across the basolateral membrane, $\Delta\mu_K$. Therefore,in normally transporting epithelia, changes of $\Delta\mu_K$ would be a primary mechanism for changing steady-state intracellular Cl^- activity,a^i_{Cl}. Gallbladders mounted in a divided chamber were superfused,at 23°C,with identical solutions containing,in mM:102.5 NaCl + KCl,and 1.0 $CaCl_2$, buffered to pH 8.2 with 5mM Tris-Cl and gassed with 100% O_2. In each experiment KCl in both bathing solutions was changed simultaneously either from 10 to 1 mM or from 5 to 0.5 mM. Double-barreled microelectrodes were used to measure intracellular K or Cl activities. Decreasing K^+ concentration in the bathing solutions increases $\Delta\mu_K$ from 51 ± 2 mV at 10 mM K^+ to 129 ± 3 mV at 0.5 mM K^+. This increased K^+ gradient is accompanied by a decrease in a^i_{Cl} from 26.5 ± 1.9 mM at 10 mM K^+ to 14.5 ± 1.2 mM at 0.5 mM K^+. With currently accepted values for basolateral P_{Cl},this decrease of a^i_{Cl} cannot be explained by electrodiffusive Cl^- exit in response to the hyperpolarization produced by decreasing external K^+ but points to a clear dependence of cellular Cl^- activity on the chemical gradient for K^+. These results strongly support the idea that coupled KCl symport,across the basolateral cell membrane,is involved in transcellular Cl^- transport by the gallbladder. Supported by USPHS AM12715, HL 23332.

M-PM-K3 PROTONATION INTERCONVERTS TRANSPORT SYSTEM TO SERVE FOR AMINO ACIDS OF UNLIKE CHARGE. H.N. Christensen & M. Makowske, Dept. of Biol. Chem., Univ. of Mich., Ann Arbor,MI 48109

Zwitterionic, anionic, and cationic amino acids usually show independent routes for membrane transport depending on molecular charge, apparent anomalies arising from transport of a species other than that anticipated. Two anomalies persisted in hepatocytes and a hepatoma line, however, despite such tests: 1. A Na^+-dependent system transporting aspartate, cysteate and cysteinesulfinate ($CySO_2^-$) in hepatocytes, but scarcely glutamate and longer anions, was unexpectedly inhibited by zwitterionic amino acids usually associated with neutral System ASC; 2. That neutral system was unexpectedly sensitive to pH lowering,pK' ca 5.8. These anomalies have a common origin:The transport activity for aspartate and its analogs is lost with rising pH along a curve reciprocal to the one by which threonine transport is gained. The low $pK_1' = 1.5$ for cysteate and $CySO_2^-$ in free solution largely excludes their protonation to zwitterionics at pH 6 and up. Threonine could scarcely become an anion in the same pH range. Hence the competition occurs between the anion and the zwitterion, with H^+ necessarily a third reactant. Under our model H^+ binds not to the anionic amino acid, but to the receptor site Z to form ^+HZ. This model is supported by the mutual competitive interactions between anion and zwitterion, with similar half-saturation levels for transport and transport inhibition at a given pH, by the similarity of the space limitations for amino acid molecules of whichever charge, and by the Na^+-dependent stimulation of threonine exodus by $CySO_2^-$ whether at pH 5.5 or pH 7.5. We now ask in what other cells does the ubiquitous System ASC show this interconvertibility? The situation is superficially analogous to the protonation of the chloride exchange site of the red blood cell to increase its comparative reactivity with sulfate (Gunn) although here we have a sharper change in more detailed molecular recognition. Support acknowledged from HD01233, NIH.

M-PM-K4 Cl-INDUCED INCREASE IN SHORT-CIRCUITING CURRENT IN THE TURTLE BLADDER. John H. Durham, Mt. Sinai Sch. Med., Department of Physiology, New York, New York 10029

In a study designed to determine if Cl reabsorption in the bladder occurred by an electrogenic process, it was found that the concomitant addition of Cl to both bathing fluids of bladders bathed in Na-free, HCO_3/CO_2,SO_4 media resulted in an increase in negative I_{sc} (neg. charges flowing to the serosa) which is not consistently equal to the rate of Cl reabsorption. Although the Cl-induced increase in I_{sc} is predicted by an electrogenic hypothesis, the lack of equality between the ΔI_{sc} and Cl reabsorption is not. The possibility arises that part or all of the Cl-induced I_{sc} is not due to the Cl-reabsorptive mechanism per se, but to an interaction between Cl and another transported ion. This possibility was addressed in the present study. It was found that the addition of mucosal Cl alone or serosal Cl alone to post-absorptive bladders bathed under the above conditions results in an increase in the I_{sc}. Mucosal Cl addition (n=18) increased the I_{sc} (uA/1.5cm^2) from -13 ±3 to -27 ±4 (k_m, 0.2 ±0.04 mM Cl, n=7). Serosal Cl addition (n=15) increased the I_{sc} from -13 ±4 to -26 ±4 (k_m, 0.7 ±0.2 mM Cl, n=7). In bladders from acidotic turtles, (NH_4Cl load), serosal Cl addition (n=14) increased the I_{sc} from -5 ±1 to -13 ±2 (k_m, 0.4 ±0.1 mM Cl, n=12), but mucosal addition produced no change. These Cl-induced increases in I_{sc} could be interpreted as an increase in luminal acidification current as depicted in a model cell containing: on the serosal membrane, a passive Cl:HCO_3 exchanger and parallel Cl and HCO_3 conductances; and on the luminal membrane, an electrogenic acidification pump in parallel with a passive Cl:HCO_3 exchanger which is inoperative in acidotic turtles.

M-PM-K5 BUMETANIDE INHIBITS MUCOSAL HCO_3 EFFECT ON APICAL MEMBRANE POTENTIAL IN NECTURUS GALLBLADDER. Lynn M. Baxendale and William McD. Armstrong,Dept.Physiol.,Indiana Univ. Sch.Med.Indianapolis, Indiana 46223.

NaCl and water movement across leaky epithelia are stimulated by HCO_3.The mechanism for this effect is not yet understood. NaCl and water are transported through transcellular and paracellular pathways. Coupled transapical NaCl entry is thought to be the rate limiting step in the transcellular path. This process is inhibited by Bumetanide.The effect of mucosal HCO_3 in the presence of Bumetanide was investigated in Necturus gallbladders.Bladders,mounted in a divided chamber,were superfused with identical control solutions containing(in mM):100 NaCl, 2.5 KCl, 1.0 $CaCl_2$, 10 Tris Cl (pH 8.2) gassed with 100% O_2.Cells were impaled through their mucosal surfaces with open-tip microelectrodes.During impalements, the mucosal control solution was changed to a similar one (pH 8.2) in which Tris-Cl was replaced by 2.4mM $NaHCO_3$ gassed with air (0.03% CO_2). In six experiments, apical membrane potential (Va) depolarized (ΔVa=5.6±1.1mV,S.E.), transepithelial potential (V_T) increased (ΔV_T=0.5±0.1mV),and transepithelial resistance (R_T) decreased (ΔR_T=17±3ohm.cm^2). These changes are significant and are consistent with HCO_3 stimulation of NaCl coupled entry and fluid absorption.Cell swelling and dilution of cellular K+ could result,thus depolarizing Va.Addition of Bumetanide (1 mM) to the mucosal control medium had no significant effect on Va or V_T. R_T increased slightly,perhaps reflecting an increase in shunt resistance due to Bumetanide inhibition of fluid absorption.Addition of mucosal HCO_3 in the presence of Bumetanide did not significantly change Va.However,V_T increased (ΔV_T=0.24±0.06mV) and R_T decreased (ΔR_T=8±2ohm.cm^2).These data suggest that Bumetanide inhibits the effects of HCO_3 on NaCl entry. Supported by USPHS AM12715,HL23332

M-PM-K6 PANCREATIC SOMATOSTATIN RELEASE OCCURS AT A LOWER GLUCOSE CONCENTRATION THAN INSULIN RELEASE. Peter Ronner and Antonio Scarpa, Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

The splenic Brockmann body of channel catfish (Ictalurus punctatus) was isolated microsurgically and perfused in vitro (Am. J. Physiol. 243 (#5), 1982) at 2mM glucose. Then it was stimulated with glucose ranging in concentration from 5 to 20mM in a random sequence. The release of insulin and somatostatin was measured by radioimmunoassay and the corresponding dose-response curves were derived. As in a typical mammalian pancreas, insulin release was stimulated half-maximally at 12mM glucose. In contrast, half-maximal somatostatin release occurred already at 4mM glucose. Thus, at 5mM glucose e.g., insulin release amounted to 2% (10 pg/min x mg tissue) of the release at 15-20mM glucose, whereas somatostatin release amounted to 60% (170 pg/min x mg tissue) of the release at 15-20mM glucose. In accordance with this finding, upon stimulation with 15 or 20mM glucose, somatostatin release usually peaked one fraction (4 min) before insulin release. The plasma glucose concentration of catfish was reported to be 3-4mM; in our fish we found it to be 6.6±1.5mM (mean±S.E.; n=10). A glucose dependence of somatostatin release similar to channel catfish was not yet reported for the mammalian pancreas. However, the overall secretory characteristics of the channel catfish pancreas are similar to those of the mammalian pancreas (Ref.). In addition, a considerable baseline somatostatin release is usually observed with mammalian pancreata perfused at 5mM glucose. Therefore, we expect that also in the mammalian pancreas D-cells respond to a lower glucose concentration than B-cells.

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M-PM-K7 LINKED FUNCTIONS IN BOVINE NEUROPHYSINS. B.A. Whittaker and N.M. Allewell, Wesleyan University, Middletown, CT 06457; E. Breslow, Cornell University Medical College, New York,NY 10021.

Bovine neurophysins I and II (NP I and NP II),the carrier proteins in the posterior pituitary for oxytocin and vasopressin, respectively, are systems in which subunit association, ligand binding, and ion binding are thermodynamically linked. We have begun to use analytical gel chromatography, reaction microcalorimetry, and potentiometry to examine these linkages and to compare NP I and NP II. LiCl, which at a concentration of 1.4M may allow a second hormone binding site to be occupied under some conditions (Nicolas et al., 1978), has been shown to have little effect at pH 5.6 on the self association of NP I (Nicolas, 1979). We find, however, that it produces a five-fold increase in $K_{Dimerization}$ for NP II in 50 mM Tris-HCl at pH 8.1. Similarly, substitution of 0.1 M KPO_4 for 0.1 M KAc at pH 5.6 increases $K_{Dimerization}$ for NP I by a factor of four. This effect appears to be smaller than that observed for NP II under the same conditions (Tellam and Winzor, 1980). On the other hand, values of $\Delta H_{Binding}$ for NP II and the vasopressin analog, Met-Tyr-Phe-NH_2, at 35°C are very similar in 0.1 M KPO_4 and 0.1 M KAc, while the corresponding values for NP I differ by approximately a factor of two. The calorimetrically determined values of $\Delta H_{Binding}$ for native NP II at pH 6.2 for a series of ten di- and tripeptide hormone analogs bracket the value for Phe-Tyr-NH_2 and nitrated NP II determined by van't Hoff analysis (Breslow and Gargiulo, 1977). The extreme values were obtained with Ala-Tyr-Phe-NH_2 (-4.5±0.3 kcal/m) and Phe-Tyr-NH_2 (-14.1±2 kcal/m). Contributions of linked proton binding to these effects are under investigation. Supported by NSF grant PCM-7920673 (to NMA) and NIH grant GM 17528 (to EB).

M-PM-K8 TRANSPORT OF GUANOSINE 3', 5' CYCLIC MONOPHOSPHATE (cGMP) INTO HUMAN ERYTHROCYTES. J. Nicotra, M. Needle, W. Ward, A. Bennun, and V. DeBari; The Renal Laboratory, St Joseph's Hosp & Med Ctr, Paterson, NJ and Depts of Zoology and Biochemistry, Cook College and the Graduate School, Rutgers University, Newark and New Brunswick, NJ

Because the human red blood cell (RBC) lacks significant adenylate or guanylate cyclase (GC) activity, transport from the extracellular fluid has been suggested as a possible mechanism for providing adenosine 3', 5' cyclic monophosphate (cAMP) and cGMP to the intracellular milieu. Recently, this has been shown to be the case for cAMP, which apparently enters the cell through anion channels. We have studied cGMP uptake using 8-^{3}H-cGMP and found that uptake from a 1 µM solution is linear over a four hour period. Uptake is also dependent on the buffer system used, bicarbonate and phosphate being roughly equivalent and tris hydroxymethyl aminomethane (tris) providing for a four-fold greater rate of uptake. The kinetics were studied according to the Michaelis-Menten scheme, and although no saturation effect was observed at concentrations up to 1 µM, tris-buffered incubations demonstrated an increasing slope at lower concentrations, suggesting that the system is subject to regulatory mechanisms. Diisothyocyanostilbene disulfonic acid (DIDS), an anion channel blocker, caused a 10% reduction in uptake at 5 µM and a 64% reduction at 10 µM. Uptake was found to be exquisitely sensitive to divalent cations, Ca^{2+} causing an 80 - 85% reduction in the range of 1 to 10 mM and Mg^{2+} causing increases up to 34% at 10 mM. No significant binding of cGMP to EDTA-treated RBC ghosts was observed. These data suggest that transport of cGMP may substitute for the activity of GC in the human RBC and that transport characteristics may be under physiological regulation.

M-PM-K9 THE INITIAL RATE AND PLATEAU LEVEL OF DOPAMINE UPTAKE BY BLOOD PLATELETS. T. NIHEI, Department of Medicine, University of Alberta, Edmonton, Canada.

Blood platelets take up biogenic amines such as serotonin and dopamine through two distinct processes. One is inhibited by imipramine and its initial rate follows the Michaelis kinetics, while the other is insensitive to imipramine. Both processes proceed to accumulate amines until the amine level in platelets reaches a plateau at a given amine concentration in medium. The plateau level of imipramine-insensitive process increases linearly with medium amine concentration, whereas that of imipramine-sensitive process is saturated at certain concentration of medium amine.

In this study, the concentration dependence of initial rate and that of plateau level of dopamine uptake by human platelets were examined. Assuming that platelets reversively accumulate dopamine in the storage sites, the imipramine-sensitive process can be represented by two-step reaction scheme. The experimental data from the measurements of initial rate and plateau level yield Km and Kapp, both of which depend on the rate constants of two reaction steps and the concentration of storage sites.

M-PM-K10 ESSENTIAL CARBOXYL GROUPS IN THE ANION EXCHANGE PROTEIN OF HUMAN RED BLOOD CELL MEMBRANES

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Studies on chloride self-exchange in human red blood cell membranes (RBCMs) as a function of extracellular pH, have indicated that the exchange at acid pH is activated by deprotonating residue(s) with an apparent pK of 5.2-5.4 (165 mM extracellular Cl^-, 0 °C). The identity of the residues is unknown, but they could be carboxyls. To adress this question, we have reacted RBCMs with a water-soluble, membrane-impermeable carbodiimide: 1-ethyl-3-(4-azonia-4,4-dimethylpentyl)-carbodiimide iodide (EAC), and studied the effects on the exchange after the reaction was stopped and the membranes washed free of unreacted EAC. The time course of irreversible transport inactivation shows a biphasic behaviour: 50 % of the exchange capacity inactivates at a rate of 0.35 min^{-1} (30 mM EAC, 165 mM KCl, pH 6, 38 °C), the remaining transport capacity inactivates at a rate at least 100-fold less. The rapid phase of inactivation is accelerated 2-fold, the slow more than 10-fold, by addition of 60 mM of the nucleophile tyrosine ethyl ester to the reaction medium. This argues strongly that the transport inactivation results from covalent modification of carboxyl groups. The carboxyl group(s) may be in the anion recognition site, as the inactivation rate was slowed about 3-fold by substituting sulphate for Cl^-. RBCMs reacted with 30 mM EAC for 32 min had an exchange capacity of 49 ± 1 % of control. These membranes were subsequently reacted with the disulphone stilbene DIDS (0 - 1.2 x 10^6 molecules/RBCM). The residual flux decreased as a linear function of added DIDS, which extrapolated to zero at 0.6 x 10^6 molecules/RBCM.

M-PM-K11 THIOCYANATE (SCN^-) INHIBITION OF Cl^- AND H^+ TRANSPORT BY GASTRIC MUCOSA. W.W. Reenstra, and J.G. Forte. Dept. of Physiology-Anatomy, Univ. of Calif., Berkeley, CA 94720.

A study of the effects of thiocyanate (SCN^-) and imidazole (IM) on acid secretion (J_H) and net chloride flux (J^{net}_{Cl}) across isolated bullfrog gastric mucosa has been undertaken. Gutknecht and Walter (BBA 685:233) postulated that SCN^- inhibits J_H by entering a restricted luminal space, of low pH, forming HSCN, and diffusing across the apical membrane. For mucosa bathed with Cl-ringers on the nutrient sice (N) and 110mM NaIsethionate (ISE), pH 8.0, on the secretory side (S), 15mM SCN^- (N) decreases J_H and J^{NS}_{Cl} by 1.90 ±.33 (S.E.) and 2.67 ±.30 uEq/hr·cm^2, respectively (P>.05). Similar inhibitions of both J^{net}_{Cl} and J_H occur with secretory SCN^-. The addition of 20mM IM (N) to SCN^- inhibited mucosa with secretory pH = 8.0, increases J_H and J^{NS}_{Cl} by 0.81 ±.28 and 0.82 ±.28 uEq/hr·cm^2, respectively, and J^{NS}_{HSCN} from 0.20 ±.02 to 0.28 ±.02 uEq/hr·cm^2. J^{NS}_{Cl}/J^{NS}_{SCN} is not altered. For Cl-free mucosa (ISE^- replacing Cl^-), SCN^- inhibition of J_H is reversed by IM (N) but the increase in J_H, 0.63 ±.10, is significantly greater than the increase in J^{NS}_{SCN}, 0.23 ±.07 (P< 0.001). As IM is postulated to increase J_H solely by increasing the luminal pH and thereby reducing [HSCN], these results are inconsistent with Gutknecht's hypothesis unless an electroneutral exchanger equilibrates SCN^- and Cl^- across the apical membrane. Changing secretory ISE to Cl^- increases J^{NS}_{Cl} (2.27x) and J^{NS}_{SCN} (1.81x). Provided the equilibration of the secretory and luminal spaces is slow, these results are consistent with rapid SCN^-/Cl^- exchange across the apical membrane. The SCN^--induced increase in transmucosal PD can be accounted for by a SCN^--induced increase in the H^+ conductance of the apical membrane. (Supported by USPHS Grant #AM10141)

M-PM-K12 NET CHLORIDE TRANSPORT ACROSS THE HUMAN ERYTHROCYTE MEMBRANE INTO LOW CHLORIDE MEDIA: EVIDENCE AGAINST A SLIPPAGE MECHANISM. Philip A. Knauf, Nancy A. Mann and Jean E. Kalwas, Dept. of Rad. Biol. & Biophys., Univ. of Rochester Med. Ctr., Rochester, NY 14642.

The anion exchange protein (band 3) can exist in two forms, one with the transport site facing the outside medium (E_o) and one with it facing the cytoplasm (E_i). Although band 3 primarily catalyzes one-for-one anion exchange, it also mediates a much smaller net anion flow. According to the slippage model, net chloride efflux can occur if the E_o form slips into the E_i conformation, which can then bind Cl^- and transport it outward. At low external Cl^-, the fraction of band 3 in the E_o form increases. This should increase net chloride efflux if it is due to slippage from E_o to E_i. Consistent with this, Frohlich et al. (J. Gen. Physiol., Jan., 1983) report that at 25°C net chloride flow increases greatly if the external Cl^- is reduced below 50 mM. We have found a similar increase in net Cl^- permeability (P_{Cl}) at 37°C, from 0.053 ± 0.006 (SEM) mmol/kg Hb-min-mM in 150 mM Cl^- to 0.30 ± 0.03 in 1 mM Cl^-. Niflumic acid (NA) is a non-competitive inhibitor of Cl^- exchange which binds preferentially to the outward-facing unloaded (E_o) and chloride-loaded (ECl_o) forms of band 3 (Knauf et al., Biophysical J. 33:49a). According to the slippage model NA should inhibit net anion transport at low external Cl^-, by preventing the change from E_o to E_i. In contrast, 50 μM NA does not decrease P_{Cl} at 1 mM Cl^-. Moreover, in 150 mM Cl^- media, NA increases P_{Cl} 3-fold (P<.0001). These data argue against the slippage model, but are consistent with a model in which net anion flow results from "transit" of Cl^- across diffusion barriers in series with the transport site. The data further suggest that the E_o and ECl_o forms of band 3 are more permeable to net chloride flow than E_i and ECl_i. (Supported by NIH Grant AM 27495.)

M-PM-K13 THIOL ACTIVATED, PASSIVE K^+/Cl^- TRANSPORT IN LOW K^+ SHEEP RED CELLS: EFFECT OF Cl^- REPLACEMENT, AND OF FUROSEMIDE. P. K. Lauf, Dept. Physiol. Duke Univ. Med. Ctr. Durham, N.C. 27710.

The SH-group reagent N-ethylmaleimide (NEM) is known to activate Cl^- dependent K^+ transport in low K^+ (LK) but not in high K^+ (HK) sheep red cells (Lauf & Theg, Biochem. Biophys. Res. Commun.92: 1980,1422). As NEM also activates passive K^+/Cl^- transport in other mammalian HK type red cells (Lauf, Adragna & Garay, J. Gen. Physiol. 80: 1982,19a), the differential reactivity of the two sheep red cell genotypes is of particular interest with respect to the origin of the LK steady state cell Here I report on the effect of Cl^- replacement by various anions, readily exchanged across band 3 protein, and on the effect of furosemide on NEM induced K^+ fluxes. Cells equilibrated in decreasing and increasing concentrations of HCO_3^- and Cl^-, respectively, showed in regard to Cl^- a convex hyperbolic activation of K^+ efflux. In contrast, activation of K^+ flux in media with increasing Cl^-/NO_3^- or Cl^-/SCN^- was concave upward up to full Cl^- replacement. Testing the effect of various inorganic anions on NEM stimulated K^+ fluxes, the following sequence of decreasing inhibitory action was seen SCN^-, NO_3^- =I^-, HCO_3^-, Cl^-. Furosemide, considered a weak anion at physiologic pH, inhibited 50% of Cl^- mediated K^+ flux at 3 mM. However, the action of furosemide was potentiated by more than an order of magnitude when the K^+ analogue Rb^+ was present in the external medium. Hence loading with Rb^+ (or K^+) of the K^+/Cl^- transporter increases its affinity for this loop diuretic. These data do not exclude the possibility that in its response to furosemide K^+/Cl^- transport in LK sheep red cells resembles that of Na^+K^+ cotransport in duck red cells to bumetanide (Haas & McManus, Biophys. J. 37:1982,214a) but seems to be independent of a Na^+ component. Supp. by NIH grant AM 28.236.

M-PM-K14 Cytochemical and Freeze Fracture Evidence for Secretion Pathways in Rat Parotid Gland. H.L. BANK and J.A.V. SIMSON, Medical University of South Carolina, Charleston, SC

Although the primary secretory fluid in salivary glands contains ions at concentrations comparable to serum, the mechanism by which water, inorganic ions and small organic ions gain access to the secretory fluid is unclear. Using a lead ion tracer method, we have found evidence that a paracellular pathway exists for the passage of inorganic ions between acinar cells, and, to some extent, between intercalated duct cells in the secretory unit of rat parotid gland. The glands were perfused briefly *in situ* with sodium acetate followed by lead acetate (1 min). After a second sodium acetate rinse, the remaining lead was precipitated by phosphate and the tissue simultaneously fixed with glutaraldehyde. The lead tracer was found to permeate readily the tight junctions between acinar cells, and occasional lead deposits were present on the luminal side of the junctional complex between intercalated duct cells. The tracer did not pass through the junctional complexes joining striated duct cells. These cytochemical findings were substantiated with freeze fracture studies which demonstrated that the zonula occludens between acinar cells had only one or two sealing strands while that between intercalated duct cells possessed 1-3 sealing strands. Thus, the permeability of the epithelial junctions to a brief infusion of lead ions was correlated (inversely) with the number of sealing strands in those junctions between cells in both the epithelium of the rat parotid gland.

M-PM-K15 TRANSPORT PROPERTIES OF THE APICAL MEMBRANE OF THE MAMMARY ALVEOLAR CELL IN THE GOAT. Margaret C. Neville, Malcolm Peaker and David Blatchford. The University of Colorado School of Medicine, Denver, CO80262 and the Hannah Research Institute, Ayr, Scotland.

Monovalent ion transport was studied in the mammary gland of conscious, lactating goats. Isotonic solutions were infused into one udder, mixed with the milk and milked out 6 hours later. The secretion or resorption of monovalent cations was determined by measuring the volumes of milk and the concentrations of ions in the milks of both udders the day prior to, the day of and the day after the experiment. From these measurements expected yields were calculated for Na, Cl, K and water and compared to the actual yield, including infusate, during the experimental period. When a volume of isotonic saline equal to one-half the expected milk yield (100-300 ml) was infused into each of 5 goats, an average of 34±2% of the infused Na, 27±7% of the infused Cl and 30% of the infused water were resorbed indicating that the mammary epithelium has the ability to resorb NaCl. K, lactose and protein yields were unchanged indicating that milk secretion was not affected. To determine whether the resorption of the two ions is obligatorily coupled, isotonic glucosamine Cl was infused into 2 animals using the same protocol; 20% of the infused Cl was resorbed. However, sodium moved in the opposite direction, secretion into the milk space being increased by 25%. When isotonic KCl was infused into 3 animals an average of 52% of the infused K and 46% of the infused Cl were resorbed; Na secretion was again increased by 30%. The results of these experiments suggest: net Na, Cl and K fluxes across the mammary apical membrane are independent of each other, sodium equlibrates passively across this membrane and Cl and K are pumped from the milk space into the cell. Supported in part by NIH grant HD 14013 and a travel grant from Burroughs Welcome Fund.

M-PM-Pos1 **KINETIC CHARACTERIZATION OF DETERGENT SOLUBILIZED SARCOPLASMIC RETICULUM (SR) ATPase.** Danka Kosk-Kosicka, Mark Kurzmack, and Giuseppe Inesi. Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Functional characterization of SR solubilized with the detergent dodecyloctaethylene glycol monoether ($C_{12}E_8$) (Dean and Tanford, Biochemistry, 17, 1683, 1978) was carried out with both steady state and rapid kinetic methods. A comparison was also made with the behaviour of the ATPase in leaky membrane vesicles. All measurements were made with very dilute protein concentrations (25 μg/mL) to favor maximal solubilization with $C_{12}E_8$. It was demonstrated by rapid quench methods that in analogy to the membranous enzyme, the catalytic mechanism of the solubilized ATPase involves early formation of a phosphorylated enzyme intermediate which then undergoes hydrolytic cleavage and release of Pi. The turnover of the solubilized enzyme is higher at intermediate ATP concentrations, and approximately equal to that of leaky vesicles at high (mM) ATP concentrations. The phosphorylated intemediate can also be formed in the reverse direction of the hydrolytic reaction by exposing the solubilized enzyme to Pi in the absence of Ca^{2+} at pH 7.5 and in the presence of dimethylsulfoxide. The ability of the ATPase to be phosphorylated, the hydrolytic activity, and the kinetic regulation of the enzyme are affected with selective patterns by the solubilization. It was demonstrated by rapid quenching that the solubilized enzyme very quickly loses its activated state upon removal of Ca^{2+} with EGTA. It is concluded that detegent solubilization yields the same number of active enzyme units as found in native membranes, but removes structural constraints of kinetic regulation. Supported by the USPHS (HL27867) and the Muscular Dystrophy Association of America.

M-PM-Pos2 COMPARISON OF TRYPTIC FRAGMENTS OF RABBIT AND HUMAN MUSCLE SARCOPLASMIC RETICULUM: A MODEL FOR DYSTROPHIC MUSCLE SARCOPLASMIC RETICULUM. B. Nagy and F.J. Samaha, University of Cincinnati Medical College, Cincinnati, Ohio

Tryptic digestion of sarcoplasmic reticulum (SR) prepared from rabbit and from human muscles was compared to assess a possible model for the assumed role of proteolytic enzymes in human muscular dystrophies where alteration of the SR protein components were observed: the decrease in the amount of 100K protein component, ATP hydrolsis by the MgCaATPase and Ca^{2+} uptake. Digestion of SR proteins with a protein: trypsin ratio of 1000 was stopped with excess soybean trypsin inhibitor. SR was sedimented in a Beckman Airfuge and both supernatant and sediment were analyzed for protein components in SDS polyacrylamide gel electrophoresis and for ATP-ase and Ca^{2+} uptake. No large peptides appeared in supernatant, all proteolytic fragments larger than 20K remained attached to SR membranes. Activities changed parallel with decrease in 100K protein content. Increase in 60K dalton and 45K dalton protein bands occurred in the first part of the digestion and after about half of 100K band reduction the two smaller bands were reduced also. There is no significant difference in digestion pattern between rabbit and human SR. At 60% reduction in 100K dalton protein the ATPase and Ca^{2+} uptake together with SDS polyacrylamide gel electrophoresis pattern was similar to that found in undigested dystrophic human SR. The results are consistent with the suggestion that increased protease activity in dystrophic human muscles may affect not only structural proteins but membrane proteins also.

Supported by the Muscular Dystrophy Association

M-PM-Pos3 THE EFFECT OF POTASSIUM IONS AT THE PROTEIN-LIPID INTERFACE IN SARCOPLASMIC RETICULUM MEMBRANES. Barry S. Selinsky and Philip L. Yeagle, Dept. of Biochemistry, SUNY/Buffalo School of Medicine, Buffalo, NY 14214.

The effects of KCl on light sarcoplasmic reticulum (LSR) and heavy sarcoplasmic reticulum (HSR) isolated from rabbit have been investigated. The ATP-hydrolysis activity of the (Ca^{++} + Mg^{++}) ATPase found in both membrane systems is activated by KCl, with maximal activation at 60 mM. The proteolysis pattern of trypsin-treated membranes is dependent upon KCl concentration. At concentration greater than 60 mM the (Ca^{++} + Mg^{++}) ATPase is primarily split into two bands of 45,000 and 55,000 daltons as determined by SDS-disc electrophoresis. At lower concentrations of salt, no fragments with molecular weight greater than 30,000 daltons are observed. Phosphorus nuclear magnetic resonance was used to investigate protein-lipid interactions in the two membrane systems. Using either egg phosphatidylcholine or lipids extracted from LSR as a reference, 10% of membrane phospholipids are motionally restricted in intact LSR in 20 mM HEPES buffer, pH 7.0. Adding 100 mM KCl increases the motionally restricted component to 20%. In 10 mM TRIS, pH 7.0, 30% of membrane phospholipids are restricted; the addition of 100 mM KCl decreases this to 10%. Using HSR in 20mM HEPES, pH 7.0, 20% of the lipid is motionally restricted independent of KCl concentration. T_1 and T_2 relaxation times for both LSR and HSR were measured and employed in the interpretation of this data. Our results indicate that either a) KCl causes a conformation change in the (Ca^{++} + Mg^{++}) ATPase which is reflected in the interaction between the protein and neighboring phospholipids, or b) KCl causes changes in the lipids which reflect on the conformation of the protein. Also, there is a significant difference between LSR and HSR with respect to protein-lipid interactions.

M-PM-Pos4 **EFFECTS OF ANESTHETICS ON THE ROTATIONAL DYNAMICS OF PROTEIN AND LIPIDS IN SARCOPLASMIC RETICULUM** *Diana J. Bigelow and David D. Thomas,* Dept. of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455

We have used electron paramagnetic resonance (EPR) to study the effects of anesthetics on the rotational motion of nitroxide spin labels attached to lipid hydrocarbon chains or to the Ca-ATPase (Ca pump) protein, correlating molecular dynamics with enzyme functions. Previous studies show that this enzyme's activity correlates well with both lipid fluidity and protein mobility and that about 20 phospholipids/Ca-ATPase are motionally restricted, indicating close association between lipid and protein. (Thomas, Bigelow, Squier &Hidalgo, 1982; Biophys. J. *37*: 217).

Enhancement of the enzyme activity without loss of coupling in the presence of 5%(v/v) diethyl ether has been reported by other workers, suggesting that the ether may alter lipid-protein interactions in such a way as to facilitate those protein motions involved in the transport of calcium. The present study probes lipid and protein dynamics in the presence of diethyl ether, correlating them with enzymatic function under the same conditions.

Nanosecond time-scale lipid motions were measured by conventional EPR (V_1) of spin-labeled derivatives of fatty acids (or their methyl esters) and phospholipids. The slower protein rotations (msec to μsec) were measured by saturation transfer EPR (V_2') of a maleimide spin label covalently bound to protein. When the enzyme activity was enhanced by 5%diethyl ether, EPR spectra showed little or no change. Similar studies were done with other anesthetics.

M-PM-Pos5 INTRINSIC DIFFERENCES IN LIPID DYNAMIC BEHAVIOR OF SARCOPLASMIC RETICULUM AND TRANSVERSE TUBULE MEMBRANES OF SKELETAL MUSCLE. Cecilia Hidalgo. Department of Muscle Research, Boston Biomedical Research Institute and Department of Neurology, Harvard Medical School, Boston, MA.

The rotational mobility of lipids in sarcoplasmic reticulum (SR) and transverse-tubule (T-tubule) membranes isolated from rabbit fast muscle was studied by conventional EPR spectroscopy. A variety of spin label stearic acid analogs with the nitroxide reporter group at position 5, 12 and 16 in the fatty acid chain were used. In the temperature range of 0-45°C, all spin labels incorporated into the T-tubule membrane yielded one-component spectra, with lower mobility of the probes than that observed in SR membranes. In contrast, fatty acid spin labels with the nitroxide reporter group at position 12 or 16 when incorporated in the SR membrane clearly revealed two-component spectra below 20°C. These results indicate that the lipids of the T-tubule membrane are motionally less heterogeneous and less fluid than those of the SR membrane, properties which are probably related to the high cholesterol content of the T-tubule membrane.

Breaks in the rotational mobility of the probes were observed at 26°C for T-tubule membranes and at 17°C for SR membranes. These changes in lipid mobilities for T-tubule and SR correlate with the observed breaks in Arrhenius plots of Ca^{2+}-or-Mg^{2+}-ATPase activity of T-tubule at 26°C and of the Ca^{2+}-ATPase of SR at 17°C, indicating that the lipid environment modulates the behavior of these two ATPases. (Supported by NIH Grant No. HL 23007).

M-PM-Pos6 THE SARCOPLASMIC RETICULUM OF SWINE WITH MALIGNANT HYPERTHERMIA HAS ABNORMAL CALCIUM-INDUCED and HALOTHANE-INDUCED CALCIUM RELEASE PHENOMENA.
S. Tsuyoshi Ohnishi* and Gerald A. Gronert**, Dept. of Hematology and Medical Oncology, Hahnemann University Medical School, Phila., PA 19102* and Dept. of Anesthesiology, Mayo Medical School, Rochester, MN 55901**

Malignant hyperthermia (MH) is believed to be a genetic disorder of calcium movements in muscle (1); therefore, it has been suspected that the SR (sarcoplasmic reticulum) in an MH patient or swine might have an abnormal response to halothane. However, definitive evidence has not yet been demonstrated. This is probably because the method to study the calcium release from the SR is not well developed. A method was developed using rabbit skeletal muscle to measure calcium-induced calcium release from the isolated SR (2). The existence of a Ca-gated calcium channel as a calcium release mechanism in SR was proposed (3,4). Using this method, the SR prepared from MH swine was shown to have abnormally high calcium-induced and halothane-induced calcium release.

(1) Anesthesiology 53:395 (1980), (2) J. Biochem. 86:1147 (1979), (3) in "The Mechanism of the Gated Calcium Transport Across Biological Membranes", p.275 (Academic Press, 1981), (4) Biophys. J. 37:219a (1982).

M-PM-Pos7 THE EFFECTS OF VALINOMYCIN (V) ON THE COMPOSITION OF THE SR IN TETANIZED MUSCLE. T. Kitazawa, A.V. Somlyo, H. Shuman and A.P. Somlyo, Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

The K accumulated by the terminal cisternae (TC) of tetanized frog muscle during tetanus is insufficient to account for the charge released in the form of Ca^{2+} (1). We wished to determine whether an increase in the K permeability of the SR membranes induced by V will cause increased uptake of K into the TC. Single frog semitendinosus muscle fibers (for 1 hr) and bundles of 20 to 40 fibers (for 2 hrs) were incubated in 5μM V (in 1% ethanol). Following V, the twitch of both types of preparations declined to 71-73%, while the tetanus was unchanged. V caused massive swelling (2-3 times in diameter) of the mitochondria and electron probe analysis of cryosections showed that they contained increased amounts of K (>.6mol/kg mito dry wt). Preliminary electron probe analysis showed that the K content was significantly higher (>.7mol/kg TC dry wt) and equivalent to the Ca^{2+} released, in V-treated, tetanized muscles, than in untreated, tetanized muscles. V did not reduce Mg uptake by the TC, nor did it alter the [Ca] remaining in the TC at the end of a 1.2 sec tetanus. V had no significant effect on the K content of the TC of resting muscles. The present findings 1) confirm the conclusion (1) that in resting muscle the $[K^+]$ in the SR and in the cytoplasm are equal and the trans-SR membrane potential (MP) is near zero; 2) argue against the possibility that charge neutralization for Ca^{2+} release is provided by co-transport of organic anions with Ca^{2+} and 3) suggest that the cause for the K^+ uptake being insufficient to compensate for the entire Ca^{2+} charge released during tetanus (in untreated muscle) is the insufficient permeability of the SR to K^+, and Ca release occurs in exchange for the entrance of protons, K, or Mg, depending on the prevailing conductance of the SR membrane. 1) Somlyo et. al., (1981) J. Cell Biol. 90: 577

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M-PM-Pos8 CALCIUM BINDING TO MONOMERIC AND DIMERIC SOLUBILIZED SARCOPLASMIC RETICULUM ATPase. S.Verjovski-Almeida and J.L.Silva. Federal University of Rio de Janeiro,Department of Biochemistry Rio de Janeiro 21910, Brazil.

Gel chromatography was used to characterize the size and the state of aggregation of sarcoplasmic reticulum ATPase solubilized by the nonionic detergent $C_{12}E_8$, and to measure calcium binding to monomeric and dimeric forms of the soluble ATPase.Using a calibrated chromatography column equilibrated with a buffer containing 40mg $C_{12}E_8$/ml it was possible to measure the Stokes radius of the soluble ATPase particle. The minimum radius of 45 Å, corresponding to a monomeric ATPase-detergent particle of about 160,000 dalton was obtained only at protein concentrations lower than 10μg/ml. Increasing the protein concentration to 50μg/ml shifted the elution position, and 70% of the protein eluted as a particle of 59-60 Å corresponding to a dimeric ATPase-detergent complex of 290,000 dalton. The ATPase has a strong tendency to associate specifically as a dimer, as a further increase of 100 fold in the protein concentration up to 5 mg/ml continued to show the elution pattern of dimeric ATPase; higher aggregates were not detected at significant proportions.The amount of calcium bound to the ATPase in the different fractions was measured with an elution buffer containing 50μM radioactive calcium. Calcium binding to the ATPase eluting in the dimer position was 9.4 ± 0.7 nmol Ca/mg protein and to the ATPase eluting in the monomer position was 17.6 ± 1.5 nmol Ca/mg protein. The monomeric ATPase binds two mol Ca/mol ATPase. In the soluble dimeric form only half of the calcium binding sites are occupied. As in the vesicular ATPase a maximal level of 8-9 nmol Ca/mg protein was found, it is suggested that half of the sites in a dimeric enzyme are occupied when passive Ca^{2+} binding is measured in the vesicles.

M-PM-Pos9 SARCOPLASMIC RETICULUM ATPase KINETICS: MAGNESIUM-DEPENDENCE OF THE Ca^{2+}-INDUCED TRANSITION. P. CHAMPEIL, M. P. GINGOLD and F. GUILLAIN, Service de Biophysique, Département de Biologie, C.E.N. Saclay, 91191 Gif-sur-Yvette Cedex (FRANCE) and G. INESI, Department of Biological Chemistry, University of Maryland Medical school, Baltimore, Maryland 21201 (U.S.A.)

At pH 7, 20°C and in the absence of potassium and magnesium, the intrinsic fluorescence rise after addition of calcium to a calcium-deprived enzyme was monoexponential. On the other hand, when the calcium-deprived enzyme was preincubated with magnesium, this fluorescence rise was clearly biphasic at high calcium concentrations. For a constant (higher than mM) magnesium concentration, the rate constant of the slow phase and the amplitude of the fast phase rose for the same range of calcium concentrations, between pCa5 and pCa4 . At pH 6, fluorescence signals were monophasic even with 20mM Mg^{2+}.

We also found that at pH 7, phosphorylation of the enzyme after simultaneous addition of calcium and ATP was faster when SR was originally calcium-deprived in the presence of magnesium. At pH 6 on the contrary, preincubation with magnesium did not influence the phosphorylation time course.

We suggest that the magnesium-enzyme complex presents one readily accessible site of relatively low affinity (10-100μM) for calcium, and that the already documented accelerating effect of ATP on enzyme isomerization is dependent on preincubation with magnesium.

M-PM-Pos10 CO-TRANSPORT OF ANIONS WITH CALCIUM IN ISOLATED SKELETAL MUSCLE SARCOPLASMIC RETICULUM. A. Chu, C.A. Tate, R.J. Bick, W.B. Van Winkle, & M.L. Entman. Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

In isolated sarcoplasmic reticulum (SR) vesicles, Ca^{2+}-chelating but non-Ca^{2+}-precipitating dicarboxylates, such as maleate and succinate, have been previously reported to stimulate ATP-dependent Ca^{2+} accumulation, resulting spontaneous Ca^{2+} release, and Ca^{2+}-dependent ATPase (CaATPase) activity (Chu, et al., J. Biol. Chem. 258, 1983, in press). [^{14}C]succinate was co-transported with $^{45}Ca^{2+}$ in equal amounts, and was Ca^{2+}-dependent. However, the rate of [^{14}C]succinate accumulation lagged behind that of $^{45}Ca^{2+}$. Release of both [^{14}C]succinate and $^{45}Ca^{2+}$ were induced by the Ca^{2+} ionophore, A23187. EGTA also induced efflux of both Ca^{2+} and succinate, but at a slower rate and in lesser amount than with A23187. The K_m and V_{max} of succinate accumulation were ~20 mM and 12 nmol/mg protein/min, with a Hill coefficient of 1.70. $^{45}Ca^{2+}$ and [^{14}C]succinate accumulation were measured in the presence of varying concentrations of both dicarboxylates, maleate and succinate. The two dicarboxylates had an additive stimulatory effect on peak $^{45}Ca^{2+}$ accumulation at low concentrations and were inhibitory beyond 40 mM. Maleate was a competitive inhibitor of succinate accumulation. The K_i of maleate inhibition of the rate of [^{14}C]succinate accumulation was ~17 mM, with a Hill coefficient of 1.75. The presence or absence of 100 mM chloride (KCl) did not influence accumulation of Ca^{2+} and succinate. The data suggest that succinate accumulation is Ca^{2+}-dependent, but occurs at a saturable divalent anion-specific site. While this carrier or channel requires Ca^{2+} transport, it may be controlled by other factors in addition to transmembrane potential (Supported by NIH grants HL 13870, HL 22856, and AHA, TX Affiliate).

M-PM-Pos11 MECHANISM OF A GATED CALCIUM-CHANNEL IN SKELETAL MUSCLE SARCOPLASMIC RETICULUM. Jeffrey L. Flick*, Emanuel Rubin* and, S. Tsuyoshi Ohnishi**, Dept. of Pathology and Laboratory Medicine* and Dept. of Hematology and Medical Oncology**, Hahnemann University Medical School, Phila., PA 19102

There are several mechanisms of calcium release from sarcoplasmic reticulum (SR) being proposed; i.e., voltage-gated, calcium-gated, caffeine-gated and, proton-gated. Through our studies on the calcium-gated channel(1), we have observed that the calcium-gated channel of isolated heavy SR preparations is also voltage-gated. The channel opens when an inside-negative potential difference across the SR membrane is induced by a cation-exchange method(2), and closes when the inside potential is made positive. Ruthenium red, which is a calcium-gated channel-blocker(1), can also inhibit the voltage-gated calcium release. This channel also serves as a monovalent cation channel. The permeability of monovalent cations is regulated by the same mechanism as that controlling the calcium release mechanism; i.e., it is (a) calcium-gated, (b) voltage-regulated, (c) enhanced by caffeine and (d) inhibited by ruthenium red or tetracaine. It is possible that calcium and monovalent cations are counter transported by a carrier-mediated mechanism. The proton-gated channel is different from this channel. It is not inhibited by either ruthenium red or tetracaine.
(1)in "The Mechanism of Gated Calcium Transport Across Biological Membranes" p 275 (Academic Press, 1981). (2) Biochim. Biophys. Acta. 587:121 (1979).

M-PM-Pos12 RADIATION INACTIVATION ANALYSIS OF CARDIAC SARCOPLASMIC RETICULUM CALCIUM PUMP PROTEIN. Brian K. Chamberlain, *Charles J. Berenski, *Chan Y. Jung and Sidney Fleischer, Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235 and *Biophysics Laboratory, Veterans Administration Medical Center, State University of New York at Buffalo, Buffalo, NY 14215.

The size of the calcium pump protein (CPP) in canine cardiac sarcoplasmic reticulum (SR) membranes was determined using classical target theory analysis of radiation-inactivation data. Samples of cardiac SR were irradiated in a frozen state by increasing doses of high energy electrons from a Van der Graaf accelerator. The loss of Ca^{++}-dependent ATPase activity and of Ca^{++}-oxalate loading with increasing irradiation dosage paralleled one another. Also, the loss of staining intensity of the CPP band (M_r approximately 110,000) in SDS-polyacrylamide gel electrophoresis correlated with the loss of ATPase and loading activities. The apparent target size is 210,000 to 240,000 daltons. We conclude that the CPP in canine cardiac SR exists as a dimer. (Supported by Grants AM 14632 and AM 13376 from the NIH and by a Grant from the Muscular Dystrophy Association of America).

M-PM-Pos13 SENSITIVITY OF SPIN LABELED SR TO ENZYME PHOSPHORYLATION IN AQUEOUS MEDIA AND IN DIMETHYL SULFOXIDE. Carol Coan*, Giuseppe Inesi, Sergio Verjovski-Almeida, Leopoldo de Meis. *University of the Pacific Dental School, 2155 Webster Street, San Francisco, CA 94115.

The iodoacetamide spin label (ISL) is highly selective, labeling one site per 100,000 Mr unit on the SR·ATPase. Components in the EPR spectrum exhibit a specific broadening on binding of substrates or inorganic phosphate (Pi). Addition of μm Ca^{2+} greatly enhances the substrate broadening, but reverses the effect of Pi. We now demonstrate that the Ca^{2+} enhancement is due to the division of the major spectral components into two components of close proximity, representing two distinct conformational states of the enzyme. This follows the reaction kinetics of ISL with the ATPase which show two reactive populations of sites when substrates and Ca^{2+} are both present, of approximately 4 nm/mg SR each. The two components exhibit random redistribution on removal and reintroduction of substrate indicating that they represent two forms of a given site.

EP formation by Pi in the absence of Ca^{2+} does not produce a two component EPR spectrum, and gives a less distinct separation of kinetic parameters. Addition of Me_2SO, however, induces the separation of the components in both the EPR spectrum and the reaction kinetics.

We conclude that the observed splitting of the EPR spectrum is related to formation of a "transition" complex of substrate with the activated enzyme, which is permissive of phosphoryl transfer in one half of the potential phosphorylation sites. This conformation is not produced directly in aqueous media by Pi, but is to a certain degree when Me_2SO is present.

M-PM-Pos14 **H^+ CONCENTRATION DEPENDENCE OF CA^{2+} INDUCED CHANGES OF INTRINSIC FLUORESCENCE IN SARCOPLASMIC RETICULUM (SR) ATPase.** Francisco Fernandez-Belda and Giuseppe Inesi. Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Changes in intrinsic fluorescence upon calcium binding to SR ATPase at neutral pH were first observed by Dupont (BBRC 71, 544, 1976) and attributed to a protein conformational change. We have measured incremental changes in intrinsic fluorescence upon titration of high affinity sites with Ca^{2+} at equilibrium and in the absence of ATP. Identical curves were obtained when incremental Ca^{2+} was added to obtain saturation of the sites, and when incremental EGTA was added to obtain Ca^{2+} dissociation from the sites. The Ca^{2+} concentration required for half maximal fluorescence change increased as the H^+ concentration was increased from pH 8.5 to pH 6.0. Plots of fluoresence change vs. pCa exhibited high cooperativity at pH 8.5 and minimal cooperativity at pH 6.0. Calcium titration curves obtained by measurements of intrinsic fluorescence were identical to those obtained by direct measurement of binding. Therefore, the observed changes of intrinsic fluorescence are a direct expression of calcium binding, independent of the degree of binding cooperativity.

Both measurements of intrinsic fluorescence and calcium binding are consistent with competition of one Ca^{2+} and one H^+ at each site, and cooperative interaction of binding domains (Hill and Inesi, PNAS 79, 3978, 1982). Supported by the USPHS (HL27867) and the Muscular Dystrophy Association of America.

M-PM-Pos15 **STUDIES OF SARCOPLASMIC RETICULUM (SR) Ca^{2+}+Mg^{2+}-ATPase NUCLEOTIDE INTERACTION WITH 2',3'-O-(2,4,6-TRINITROPHENYL) ADENOSINE NUCLEOTIDES (TNP-ATP, -ADP AND -AMP).** Robert Nakamoto and Giuseppe Inesi, Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, MD 21201.

Interesting properties of TNP-nucleotides are the slight enhancement of their fluorescence upon binding to SR ATPase, and the 2 to 8 fold enhancement upon addition of ATP (Watanabe and Inesi, JBC, 257, 11,510, 1982). We find that enhancement by ATP is related to the formation of phosphorylated enzyme intermediate, when ATP is added, as 1) enhancement is Ca^{2+} dependent when ATP is added and 2) enhancement can also be obtained when the enzyme is phosphorylated with Pi in the absence of Ca^{2+}. Fluorescence enhancement by addition of 50 uM ATP is much greater for TNP-AMP (7.2 fold) than for TNP-ATP (2.5 fold). This may be consistant with a better fitting of TNP-AMP in the phosphorylated active site.

We found that, independent of the presence of ATP and/or Ca^{2+}, tryptophan excitation energy (290 nm) is transferred to bound TNP-nucleotides. This effect is revealed by TNP-ATP, and even more by TNP-AMP. In addition, a discrete fluorescence enhancement is observed at 525 nm (TNP- nucleotide emission peak) when tryptophan is excited. This indicates that at least one tryptophanyl residue is near the active site; most likely Trp 227 of Fragment 3 (near Lys 190 reactive to FITC: Mitchinson et al, FEBS Letters, 146, 87, 1982), which is the only tryptophanyl residue in the sequenced (and extramembranous) segments of the ATPase protein. Other tryptophanyl residues known to be in the non-sequenced (intramembranous) protein segments are less likely to participate in the observed energy transfer. Assuming that the transfer is limited to the Trp 227, our findings indicates that this residue is very close to the TNP moiety of the nucleotide analogue. Supported by the USPHS (HL 27867) and the Muscular Dystrophy Association of America.

M-PM-Pos16 GTP-INDUCED CALCIUM ACCUMULATION IN ISOLATED CANINE CARDIAC SARCOPLASMIC RETICULUM NOT REQUIRING GTP HYDROLYSIS. C.A. Tate, R.J. Bick, W.B. Van Winkle, and M.L. Entman. Section of Cardiovascular Sciences, Depts. of Medicine and Physiology, Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030.

In canine cardiac SR, GTP is a substrate for the NTPase with a similar hydrolysis rate and substrate affinity as ATP. The GTPase represents a different enzyme cycle than the ATPase in that there is no acylphosphate formation; it is calcium independent; and there is no concomitant transport of calcium or oxalate to the intravesicular, EGTA-inaccessible space. In contrast, GTP does induce calcium accumulation (15-25 nmol/mg protein) into a compartment from which calcium is released by the ionophore, A23187, so that the accumulated calcium is not merely bound to superficial binding sites. ATP and its analogue, AMP-P(NH)P, non-competitively inhibit GTPase activity in the presence of calcium with a K_I of 1 μM and 4-8 μM, respectively; the GTP analogue, GMP-P(NH)P, competitively inhibits GTPase activity (K_I of 40-80 μM). Despite this, both analogues at 1 mM do not inhibit GTP-induced calcium accumulation. N-ethylmaleimide (NEM), which inhibits both CaATPase activity and ATP-dependent calcium accumulation, does not inhibit GTPase activity, either in the presence or absence of calcium. In contrast to GTPase activity, NEM completely inhibits GTP-induced calcium accumulation. This suggests that the binding of GTP to the NTPase induces a conformation allowing calcium accumulation into an intermediate compartment. This calcium accumulation does not require GTP hydrolysis, but is inhibited by NEM. (Supported by NIH grants, HL 13870 and HL 22856, and AHA, TX affiliate.)

M-PM-Pos17 EXTRACTION OF THE SPANNING AND ANCHORING PROTEINS OF THE TRIAD JUNCTION OF SKELETAL MUSCLE. A.H. Caswell, Dept. of Pharmacology, Univ. of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

The triad junction of skeletal muscle contains a spanning protein which crosses the gap between the terminal cisternae (TC) and transverse (T) tubules; it may also contain anchoring proteins which tie the spanning proteins to the membranes and form the junctional complex. An intact triad of vesicles composed of a T-tubule apposed to two TC vesicles was prepared from rabbit back muscle. We have previously identified the spanning protein of the junctional triad as containing a subunit doublet of Mr = 300 K and 270 K. Treatment of the triadic vesicles with Triton X 100 extracts nonjunctional membrane but leaves the spanning protein intact in the junctional complex. The whole junctional complex dissolves in a zwitterionic detergent in the presence of hypertonic KCl or NaCl but not K gluconate. The spanning protein of the triad was obtained app. 50% pure by molecular seive chromatography. The protein has an aggregate MW. of app. 1.5×10^6 daltons and contains three subunits of Mr = 300 K, 270 K and 140 K. The subunit ratios vary in different preparations and hence may represent "isozymes" rather than individual units of a single complex. A limited number of other major protein constituents of the junctional complex are also present in the triadic complex and can be extracted by detergent/NaCl. Intrinsic proteins associated specifically with the T-tubules may include the anchoring protein which holds the spanning protein in contact with the T-tubules. (Supported by NIH grant AM 21601).

M-PM-Pos18 IDENTIFICATION OF T-TUBULES/JUNCTIONAL SARCOLEMMA IN CARDIAC MICROSOMES N.R. Brandt, J.-P. Brunschwig and F.A. Lattanzio, University of Miami School of Medicine, Dept. of Pharmacology, P.O. Box 016189, Miami, Fl. 33101.

Microsomes from rabbit ventricular muscle were separated into 2 populations on continuous sucrose gradients. Vesicles banding at 22% (w/w) sucrose entrapped ouabain and bound muscarinic and β- adrenergic ligands specifically. These markers identify the band as external membrane (ExM). The bulk of the protein banded at 32% sucrose. Ca-Mg ATPase and Ca-stimulated hydrolysis of chromogenic substrates indicated that this heavier band was predominantly sarcoplasmic reticulum (CSR). Earlier experiments had demonstrated that skeletal muscle triads could be reformed from isolated T-tubules and terminal cisternae (TC) in the presence of K cacodylate. The isopycnic point of cardiac ExM altered from 22% sucrose to the buoyant density of the SR after they had been incubated with CSR or TC of skeletal muscle in the presence of K cacodylate, indicating reformation of dyads. Rejoined cardiac dyads could be disrupted into the ExM and CSR components by passage through the French press. The isolated organelles, the rejoined cardiac dyads and the mixed cardiac-skeletal complexes were visualized by electron microscopy. The ExM fraction of cardiac microsomes is composed of T-tubules and/or junctional sarcolemma.

(Supported by grants NIRA HL 28804, AM 21601 and Training grant HL 07188 from NIH).

M-PM-Pos19 CAFFEINE CONTRACTURE IN TOAD PACEMARKER PREPARATIONS. Caputo, C., Morales, P.*, López, J.R., Bolaños Pura*. Centro de Biofísica y Bioquímica, IVIC, Apdo. 1827, Caracas, VENEZUELA.
SA and AV pacemarkers were dissected from the tropical toad Leptodactylus insularis. In regularly beating pacemarker preparations, caffeine (1-30 mM) added to normal Ringer (2.8 mM K^+) induced a marked potentiation (positive inotropic effect) on the spontaneous contractile activity without evoking contracture responses at 20°C. Caffeine contractures were only elicited when the drug was added after the spontaneous relaxation that followed a K^+ contracture in low Na solution. These caffeine contractures were transient and their strength could be modulated by the K^+ and caffeine concentrations. No caffeine responses were observed when the K^+ contracture solution contained a high [Na^+] (>50mM). The ability of caffeine to induce a contracture after the spontaneous relaxation of responses initiated by sodium-free solutions ruled out the possibility that these contractures are related with depolarization of the muscle membrane. This observation might indicate that caffeine is acting on intracellular calcium pool which may be loaded during the relaxation phase of a K^+ contracture induced in low [Na], when the Na-Ca exchange is not operative. The fact, that caffeine could elicit contractures in the virtual absence of extracellular calcium, support the idea that caffeine releases calcium from intracellular sites. It is possible that normally the removal of calcium from the myoplasms after a K^+ contracture depends mainly on the Na-Ca exchange mechanism. But once that such mechanism has been blocked (low Na^+) there are intracellular structure which may be able to transiently take up Ca^{2+} from the cytoplasm.
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M-PM-Pos20 VANDATE-INDUCED CRYSTALLIZATION OF SARCOPLASMIC RETICULUM INTRAMEMBRANE PARTICLES
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Sarcoplasmic reticulum (SR) membranes isolated from rabbit skeletal muscle appear in freeze-fracture as 0.1-0.2 μm vesicles. The concave fracture surface (P-face) contains a dense population of 8.5 nm particles arranged at a center-to-center distance of about 10 nm without detectable order. The particles were previously identified as the Ca++-transport ATPase. The convex surface (E-face) is mostly smooth, displaying an occasional particle but never complementary arrays of pits. By incubating the vesicles (1 mg SR protein/ml) at 4°C in 0.5 mM EGTA, 10 mM imidazole, 0.1 M KCl (pH 7.4) solutions containing 5 mM sodium vanadate (Na_3VO_4), a powerful inhibitor of the Ca++, Mg++-ATPase, several structural changes occur. Most vesicles elongate into tubules 60-80 nm in diameter and the 8.5 nm intramembrane particles of the P-face become regularly organized into parallel rows. The particle rows are coiled around the tubules in left-handed helices, the axis of rows forming a 50-60° angle with the long axis of the tubules. The particles repeat along the rows at 8.5-8.8 nm and the rows repeat at 9.0-9.5 nm. Parallel furrows are clearly visible on the (convex) E-face of the tubules. Frequently, the furrows are resolved into rows of pits, complementary images of the particle rows. These data suggest that vanadate induces a conformational change in the Ca++- transport ATPase which causes crystallization of the intramembrane particles.
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M-PM-Pos21 Lateral Phase Separation in Sarcoplasmic Reticulum Membranes. Barry S. Selinsky, Arindam Sen, S.W. Hui and Philip L. Yeagle, Dept. Biochemistry, SUNY/Buffalo School of Medicine, Buffalo, NY 14214 and Dept. Biophysics, Roswell Park Memorial Institute, Buffalo, NY 14203.

The possibility of extensive lateral phase separation of membrane components in biological membranes has been examined only very recently. Dehydration has been shown to cause phase separation of lipids in retinal rod outer segment membranes (Greiner, et al., Biophys.J. 39:241 (1982)) and in sarcoplasmic reticulum (Crowe and Crowe , Arch. Biochem. Biophys. 217: 582(1982)). Similar lipid phase separation has also been observed in chloroplast thylakoid membranes on heating to 50°C (Younaris, et al.,Biophys. J. in press). We have examined sarcoplasmic reticulum (SR) to determine if less extreme conditions can cause a lateral phase separation. ^{31}P NMR and freeze fracture electron microscopy. Freshly isolated SR appears to be entirely composed of phospholipids in lamellar phase as determined by Sarcoplasmic reticulum exists as unilamellar vesicles with protein randomly dispersed throughout the lipids. Incubating SR at 37°C for 48 h results in aggregation of the sample into visible "clumps". The ^{31}P NMR spectra of this material includes a large isotropic peak indicative of phase separation of the phospholipids. Freeze fracture EM confirms that a gross phase separation has occurred. Isotropic NMR resonances do not appear in lipid extracts of SR. These results may be indicative of an interesting instability in the SR membrane.

M-PM-Pos22 CHARACTERIZATION OF TRANSVERSE TUBULAR MEMBRANES USING ONE AND TWO DIMENSIONAL ELECTROPHORESIS. Vincent R. Okamoto, Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

Purified transverse tubular membranes (TT) isolated from chicken skeletal muscle exhibit distinct electrophoretic protein distributions when compared to purified sarcoplasmic reticulum (SR) and enriched sarcolemmal (SL) membranes. One dimensional SDS-PAGE shows that both the TT and SR possess a 102 K dalton polypeptide, the SR species being the well characterized Ca,Mg-ATPase. However, the TT displays a different isoelectric point than the SR protein on two dimensional isoelectric focusing - SDS gels suggesting that the 102K polypeptides of TT and SR are dissimilar. Autoradiograms of gels incubated in ^{125}I-WGA indicates that the TT polypeptide is a WGA positive glycoprotein whereas the Ca,Mg-ATPase is not. Evidence from detergent solubilization studies suggests the 102K TT polypeptide may represent the catalytic component of a Mg-ATPase which is specifically associated with TT membranes.

Several low molecular weight polypeptides characteristic of TT have also been identified on SDS-PAGE and two dimensional non-equilibrium pH gels. These TT components co-migrate with glycolytic and other soluble enzymes (aldolase, enolase, CPK, GPDH) present in high speed supernatants of muscle homogenates. It was not possible to extract these "soluble" enzymes by sonicating them in the presence of high ionic strength buffers containing EDTA. They could only be released by making the TT leaky by treating them with non-ionic detergents in concentrations below the CMC for the membranes. These data suggest that the low molecular weight proteins may be tightly bound specifically to TT membranes.

M-PM-Pos23 THE DISTRIBUTION OF ATPASE ACTIVITIES IN PURIFIED TRANSVERSE TUBULAR MEMBRANES. Roger Sabbadini and Vincent Okamoto, Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

Vesiculated fragments of transverse tubules (TT) and sarcoplasmic reticulum (SR) membranes were purified from heterogeneous microsomal membrane fractions of chicken breast muscle by a modification of an iterative calcium-oxalate loading technique. The distribution of ATPase activities were determined for the TT and SR and were compared to enriched fractions of sarcolemmal (SL) membranes. The TT were characterized by high rates of Mg-ATPase (232 $\pm$ 28 um/mg/hr) but were virtually devoid of Ca,Mg-ATPase (8.8 $\pm$ 2.7 um/mg/hr) activity. Moderate levels of a latent Na,K-ATPase were observed for TT membranes when unmasked with valinomycin and monensin. In contrast to TT membranes, highly purified SR membranes displayed an active Ca,Mg-ATPase but negligible Na,K-ATPase and Mg-ATPase. The lack of significant Mg-ATPase activity in the SR and SL fractions suggested that the Mg-ATPase was uniquely associated with the TT membranes. The Mg-ATPase of the TT was insensitive to inhibition by sodium azide and oligomycin at concentrations shown to exert maximum inhibition of the F_1ATPase. The Mg-ATPase was also resistant to the effects of ouabain and orthovanadate. The Mg-ATPase displayed temperature and pH optima (25°C, pH 7.3) which were distinguishable from the Ca,Mg-ATPase (45°C, pH 7.0) of highly purified SR fractions but which were very similar to the temperature and pH dependencies of the Mg-ATPase observed for the mixed microsomal fractions from which the TT membranes were derived, suggesting the Mg-ATPase often seen in crude SR fractions may originate from TT contamination. These data indicate that the Mg-ATPase is distinguishable from other ATPases and may therefore be of value as a specific biochemical marker for TT membranes.

M-PM-Pos24 ROTATIONAL DYNAMICS OF PROTEIN AND LIPID IN DOG CARDIAC SARCOPLASMIC RETICULUM Jeffrey K. Lu, Charles F. Louis, and David D. Thomas. Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455.

The major enzyme which controls the cytoplasmic concentration of calcium during muscle contraction is the membrane-bound Ca-ATPase (calcium pump) of sarcoplasmic reticulum (SR). Protein and lipid rotational motions, as monitored by electron paramagnetic resonance (EPR), have been correlated with enzymatic activity (Thomas et al., Biophys. J. 37: 217-25, 1982). In the present study, we have extended this approach to dog cardiac SR. We have covalently and specifically attached a maleimide nitroxide spin label to the 100,000-dalton Ca-ATPase of dog cardiac SR and characterized its motions using conventional and saturation transfer EPR spectroscopy. After labeling, more than 90% of the probes were attached to the Ca-ATPase protein, with 70-80% retention of activity. By comparing the saturation transfer EPR spectra of spin labeled dog cardiac SR with that of rabbit skeletal SR, we found that the slow (microsecond) motions of the Ca-ATPase were significantly slower in dog cardiac SR than in rabbit skeletal SR. When a fatty acid spin label was inserted into the lipid matrix of either dog cardiac or rabbit skeletal SR, we detected no differences in the motion of the spin label. Thus, the difference in the protein mobility between the two SR preparations does not appear to be due to a difference in the fluidity of the lipid environment, but is more likely due to differences in the protein itself, such as the site labeled, protein conformation, or protein-protein interactions.

M-PM-Pos25 THE EFFECT OF IONIC STRENGTH ON THE ACTIVATION PROPERTIES OF Ca^{2+}-ATPase FROM SARCOPLASMIC RETICULUM. Therese Wiedmer and Rodney L. Biltonen, Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22908.

The enzymatic activity of Ca^{2+}, Mg^{2+}-ATPase in sarcoplasmic reticulum (SR) from rabbit muscle increases with time upon incubation at 37°C in buffer containing 20 mM $MgCl_2$. This increase in activity results in a simultaneous irreversible loss in the ability to accumulate Ca^{2+}. No such activation occurs in SR prepared in the presence of dithiothreitol (SR(+DTT)) in high salt buffer. However, incubation at 37°C in a low salt buffer containing 20 mM $MgCl_2$ causes a severalfold increase in ATPase activity. Ca^{2+} (50 μM), ATP (5 mM) or lowering the pH to 6 prevent activation by Mg^{2+} and SR membranes remain tight to Ca^{2+}. Mg^{2+}-activated ATPase shows increased susceptibility to selective modification by N-ethylmaleimide (NEM) as measured by a faster loss in enzyme activity. On the other hand, ATPase uncoupled by the ionophore A23187 is inactivated by NEM at the same rate as (fully coupled) control enzyme. We conclude that it may be important to include DTT and high salt ($\geq$ 0.6 M) in the preparation of SR membranes in order to get a stable ATPase. The fact that the two substrates Ca^{2+} and ATP prevent Mg^{2+}-activation and the experiments with NEM suggest that Mg^{2+} interacts directly with the enzyme, causing a conformational change of the ATPase which results in an increased Ca^{2+}-permeability of the membrane. (Supported by NIH grant GM-26894).

M-PM-Pos26 RECONSTITUTION OF PHOSPHOLAMBAN INTO THE SARCOPLASMIC RETICULUM CALCIUM TRANSPORT SYSTEM

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Cardiac sarcoplasmic reticulum Ca^{2+}-Mg-ATPase activities (calcium transport and ATP hydrolysis) are stimulated by phosphorylation of the membrane by c-AMP or calmodulin dependent systems. A 22K dalton protein, phospholamban, has been identified as the substrate and implicated in this activation process. Our results suggest a differential role for phospholamban in the ATPase functions. The phosphorylation status can be correlated with the levels of calcium uptake. (Ambudkar, I.S., and Shamoo, A.E., 1982, EBEC Reports, LBTM, CNRS Ed., pp39-40).

We have previously reported the isolation of phospholamban from canine sarcoplasmic reticulum (Bidlack, J.M., Ambudkar, I.S., and Shamoo, A.E., 1982, J. Biol. Chem. 257: 4501-4507). Addition of isolated phospholamban to cardiac sarcoplasmic reticulum vesicles results in a 25% stimulation of calcium uptake. This is expressed in terms of initial rates of the process. Incorporation of the 22K protein into skeletal sarcoplasmic reticulum vesicles produced effects opposite to those seen in the case of the cardiac membranes. Addition of the isolated protein induced a small but significant inhibition in the rates of calcium uptake. This inhibition could be reversed by c-AMP dependent phosphorylation.

M-PM-Pos27 OXIDATION OF REACTIVE SULFHYDRYL GROUPS OF SARCOPLASMIC RETICULUM ATPase

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The role of reactive sulfhydryl groups of sarcoplasmic reticulum (SR) ATPase has been investigated. Incubation of SR ATPase with 17 mol o-iodosobenzoic acid (IOB) per mol ATPase results in a 15% inhibition of Ca^{2+} uptake with only a 5% loss of ATPase activity. When SR ATPase is treated with 15 mol $KMnO_4$ per mol ATPase, Ca^{2+} uptake is completely inhibited. From the measurement of remaining SH groups using 5,5'-dithiobis-(2-nitrobenzoic acid), it is found that the oxidation of approximately four SH groups per ATPase molecule with $KMnO_4$ leads to a complete loss of Ca^{2+} uptake, while the oxidation of five SH groups per ATPase with IOB results in only 15% inhibition of Ca^{2+} uptake. No significant reactivation is observed after the treatment of $KMnO_4$-oxidized SR ATPase with reducing agents. The results of amino acid analysis indicate that $KMnO_4$ oxidizes the reactive SH groups to sulfonic acid groups. Among the five IOB-reactive SH groups, at least one shows a distinct Ca^{2+} dependence. Addition of IOB to the reaction medium containing $KMnO_4$ does not increase the number of oxidized SH groups, indicating that both IOB and $KMnO_4$ oxidize the same SH groups of the enzyme. $KMnO_4$ inhibition is likely to be due to the introduction of a negatively charged species, $-SO_3^-$, in the proximity of an active site. The different effects of two oxidizing agents on SR ATPase eliminate the possibility of direct involvement of SH group(s) in the ATPase reaction.

M-PM-Pos28 THE STRUCTURAL EFFECTS OF CAFFEINE ON MUSCLE: A RAPID FREEZING STUDY. T. Yoshioka. (Intr. by Henry Shuman). Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, PA.

Rapid cooling of frog striated muscles exposed to subcontractile concentrations (less than 2mM) of caffeine results in the so-called "rapid cooling contracture" (RCC) (1). In muscles fixed conventionally with glutaraldehyde-osmium, the sarcoplasmic reticulum (SR) was swollen during tension development induced by RCC (2), but liquid fixatives can induce artifactual swelling of the SR, as demonstrated by rapid freezing techniques (3,4). The present study was designed to determine whether SR swelling can also be observed in rapidly frozen freeze-substituted muscles following RCC. Frog muscle (EDL-IV) bundles were rapidly frozen in supercooled Freon 22 (-165°C.) at the time of peak tension development (60-80% of tetanus) during RCC, freeze-substituted in dry acetone and embedded. The extent of shrinkage due to tissue processing was estimated from the A-band length and indicates approximately 14% shrinkage. Diameters of the TC ($x10^{-2}\mu m$) corrected for shrinkage, were: 9.6+2.2 (n=309) 10.6+2.24 (2mM caffeine, n=213) and 12.7+2.85 (RCC with 2mM caffeine, n=325). The diameter of the TC after RCC was significantly higher than in normal controls or caffeine-treated muscles. However, the increase in TC diameter following RCC was less in freeze-substituted than in glutaraldehyde-fixed preparations ($\sim 30x10^{-2}\mu m$). Further experiments will test the structural effects of high concentrations of caffeine that produce contracture without cooling, and the compositional changes induced in the SR by caffeine. The present findings suggest that conventional glutaraldehye fixation results in serious overestimates of the amount of swelling caused by RCC. 1) Sakai, et al. (1971) Amer. J. Physiol. 220: 712-717; 2) Yoshioka, et al. (1981). Jpn. J. Physiol. 31: 29-42; 3) Somlyo, et al. (1977). J. Cell Biol. 74: 828-857; 4) Franzini-Armstrong, et al. J. Physiol. 283: 133-140. (Research supported by HL15835 to the Pennsylvania Muscle Institute.)

M-PM-Pos29 CYTOPLASMIC Ca IN RELAXED AND CONTRACTED SMOOTH MUSCLE: ELECTRON PROBE (EPA) AND ELECTRON ENERGY LOSS ANALYSIS (EELS). M. Bond, A.V. Somlyo, H. Shuman and A.P. Somlyo. Penna. Muscle Inst., Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

Total cytoplasmic Ca was measured by EPA and EELS in freeze-dried cryosections from strips of rabbit portal vein rapidly frozen at rest or during a prolonged (40 min) maximal contraction (80mM K + 10μg/ml NE). Quantitation of [Ca] by EPA in the presence of high [K] was improved by fitting the experimental spectrum to the 1st and 2nd derivatives of the K peak, to compensate for any shifts in detector calibation. EPA of cytoplasmic Ca in contracted strips (2 rabbits; 38 cells) showed [Ca] of 2.1+0.2 +S.E.M. (n=61) mmoles Ca/Kg dry wt. This was significantly higher ($P<0.001$) than [Ca] in the control muscles: -0.4+0.3 S.E.M. (n=28). Ca $L_{2,3}$ edge (~350eV) spectra of contracted smooth muscle, compared with spectra of Ca standards, indicated cytoplasmic [Ca] of 0.6-3mmol/kg dry wt. The resolution of EELS (~2eV) precluded overlap of the Ca edge with the K $L_{2,3}$ edge (~300eV). In comparison, [Ca] in the I-band of resting frog skeletal muscle is 3mmol/kg dry wt, rising to ~8mmol during a tetanus (1). In rabbit iris sphincter muscle, localized high [Ca] was found in regions consistent with central and junctional SR (jSR). JSR was readily distinguished from extracellular space in unstained cryosections by incubation with 30mM $LaCl_3$. No Ca accumulation by mitochondria in resting or contracted smooth muscle was found, agreeing with previous studies (2). These results suggest that (a) the amount of calmodulin found in smooth muscle may be insufficent to account for total cytoplasmic Ca measured in the contracted muscle and (b) Mg and not Ca is probably the major cation bound to vascular smooth muscle actin in situ, given 15.8mmoles divalent cation bound/kg thin filament dry wt. (3). (1) Somlyo, A.V. et al. (1981) J. Cell Biol. 90: 577; (2) Somlyo, A.P. et al. (1979) J. Cell Biol. 81: 316; (3) Kitazawa, T. et al. (1982) J. Mus. Res. Cell Motil. in press. Supp. by HL15835 to the Penna. Muscle Inst.

M-PM-Pos30 THE EFFECTS OF VALINOMYCIN (V) ON THE COMPOSITION OF THE SR IN TETANIZED MUSCLE. T. Kitazawa, A.V. Somlyo, H. Shuman and A.P. Somlyo, Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

The K accumulated by the terminal cisternae (TC) of tetanized frog muscle during tetanus is insufficient to account for the charge released in the form of Ca^{2+} (1). We wished to determine whether an increase in the K permeability of the SR membranes induced by V will cause increased uptake of K into the TC. Single frog semitendinosus muscle fibers (for 1 hr) and bundles of 20 to 40 fibers (for 2 hrs) were incubated in 5μM V (in 1% ethanol). Following V, the twitch of both types of preparations declined to 71-73%, while the tetanus was unchanged. V caused massive swelling (2-3 times in diameter) of the mitochondria and electron probe analysis of cryosections showed that they contained increased amounts of K (>.6mol/kg mito dry wt). Preliminary electron probe analysis showed that the K content was significantly higher (>.7mol/kg TC dry wt) and equivalent to the Ca^{2+} released, in V-treated, tetanized muscles, than in untreated, tetanized muscles. V did not reduce Mg uptake by the TC, nor did it alter the [Ca] remaining in the TC at the end of a 1.2 sec tetanus. V had no significant effect on the K content of the TC of resting muscles. The present findings 1) confirm the conclusion (1) that in resting muscle the $[K^+]$ in the SR and in the cytoplasm are equal and the trans-SR membrane potential (MP) is near zero; 2) argue against the possibility that charge neutralization for Ca^{2+} release is provided by co-transport of organic anions with Ca^{2+} and 3) suggest that the cause for the K^+ uptake being insufficient to compensate for the entire Ca^{2+} charge released during tetanus (in untreated muscle) is the insufficient permeability of the SR to K^+, and Ca release occurs in exchange for the entrance of protons, K, or Mg, depending on the prevailing conductance of the SR membrane. 1) Somlyo et. al., (1981) J. Cell Biol. 90: 577

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M-PM-Pos38 OSCILLATORY CURRENTS IN AGGREGATES OF NEONATAL RAT HEART CELLS.

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Oscillatory currents (I_{os}) are observed in aggregates of neonatal rat heart cells under voltage clamp, when 10^{-4} M ouabain is added or when K^+ is omitted from the bathing solution. I_{os} disappears again when Ca^{++} is omitted or when 3 mM Mn^{++} is added, which experiments indicate that Ca^{++} is involved in I_{os}. I_{os} resembles TI or I_{os} reported by other authors, although the period of the oscillations is much shorter.

I_{os} is most prominent after repolarizing clamp steps; amplitude and period depend on the potential to which the clamp steps are made. I_{os} is always present as a damped oscillation. The damping, expressed as the ratio between the amplitudes of two successive half periods, decreases at an increase in duration or amplitude of the preceding depolarizing pulse.

I_{os} does not disappear when 5 mM Ba^{++} is added and it does not reverse sign at the estimated E_K, indicating that I_{os} is not a Ca^{++}-activated K^+ current. Conductance measurements show, that I_{os} is not accompanied by measurable changes in conductance. Therefore it can be concluded that I_{os} is caused by oscillatory changes in driving force.

Close inspection of current tails shows, that before blockade of the Na-K pump outward current tails are preceded by a short lasting inward current tail, which can be attributed to slow inward current (I_{si}). After blockade these current tails seem to be incorporated in I_{os}. This indicates that I_{si}-like current tails are caused by the same mechanism, which causes I_{os}.

M-PM-Pos39 UPTAKES OF CALCIUM ANTAGONISTS INTO MUSCLES. David C. Pang and Nick Sperelakis, Dept. Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Vogel et al. (1979) reported that bepridil exerted an effect internally as well as its effect on blocking Ca^{++} entry into cardiac muscle. Therefore, the uptakes of tritiated verapamil, bepridil, nitrendipine, nifedipine and diltiazem by rabbit papillary muscle, chick embryonic ventricular muscle, cat ileal smooth muscle, and rat extensor digitorum longus were investigated. The uptakes of verap, bep, nitrend by muscles were much higher than those of nifed and dilt. The order of uptakes was: bep > nitrend = verap >> nifed > dilt, which corresponds to their lipid solubility. The amount of Ca antagonist accumulated by a muscle was not related to its ability to inhibit Ca^{++} uptake, since nifed and dilt were more potent in depressing Ca^{++} entry, but had the smallest uptakes. The Ca antagonists were more potent in depressing Ca^{++} uptake into smooth muscle than into cardiac muscle. The calculated internal drug concentration at steady state for bep, nitrend and verap was much higher than that in the medium; e.g., internal bep concentration was 20-fold higher than that externally. The uptakes of these Ca antagonists were probably due to passive binding to numerous intracellular sites, e.g. the SR. The small "uptake" of dilt and nifed may merely reflect binding to the surface membrane. Hence, verap, nitrend and bep enter and accumulate in the muscle cells. The ability of some of the drugs to enter the cells confers the possibility that they may exert secondary actions on internal sites, e.g. the SR, or that they may block the slow channel from the inner surface. (Supported by grants from Wallace Laboratories and the American Heart Association, Virginia affiliate.)

M-PM-Pos40 THE SODIUM PUMP IN VASCULAR SMOOTH MUSCLE. P. K. Rangachari and E. E. Daniel. Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

To assess overall Na^+ pumping in vascular smooth muscle, we have measured the number of pump sites (using ^{3}H-ouabain) and the rate of Na^+ pumping (using ouabain-sensitive ^{204}Tl uptake) in canine iliac arteries incubated in K^+-free solutions. "Specific" ouabain binding (defined as binding inhibited by 1 mM external ouabain) had a Kd of 28.6 ± 0.62 nM (n = 3). Binding was sensitive to temperature and reduced by loading tissues with lithium. External K^+ (120 mM) markedly inhibited (85%) "specific" ouabain binding at low concentrations of external glycoside (<200 nM). In 5 experiments we noted that the arteries bound 69.45 ± 8.30 pmoles/gm (external ouabain concentration being 10^{-7}M). Under comparable conditions in the same arteries, ouabain-sensitive Tl uptake was 0.1681 ± 0.0147 μmoles/gm/min. We estimate that each pump site transports on the average 24.20 Tl^+ per min. and given the customary stoichiometry of 3 Na:2K (= 2Tl), this averages to 3630 Na^+ per site min. These values are similar to those reported for a variety of tissues using other approaches (1, 2, 3). The techniques used provide a means of assessing Na-pump activity in intact arteries.

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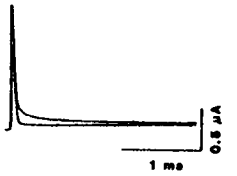
M-PM-Pos41 ACTIVE DIFFERENCE SPECTRUM OF SKINNED SKELETAL MUSCLE FIBERS STAINED WITH A VOLTAGE SENSITIVE DYE. Philip M. Best. Department of Physiology and School of Medicine-UC, University of Illinois, Urbana, Illinois 61801.

Absorption spectra were recorded from skinned (sarcolemma removed) skeletal muscle fibers stained with the voltage sensitive dye NK 2367. A computer interfaced fast spectral scanner recorded individual spectra (550 to 750 nm, 1.5 nm per point) within 30 msec. Fibers were exposed to solutions containing either 4 or 90 mM K^+ with the $K^+ \times Cl^-$ product kept constant. Potassium concentration gradients (and, by inference, voltage gradients) were imposed across intracellular membranes by making rapid exchanges between these two solutions following a period of equilibration. Difference spectra were calculated from spectral scans recorded just before and 4 seconds after the solution change. Difference spectra recorded following solution changes in which the $[K^+]$ was kept constant were flat but negatively offset from zero presumably due to dye washout or bleaching. Spectra recorded following $[K^+]$ changes were corrected for this effect. Following a 22.5 fold decrease in $[K^+]$ (90 → 4 mM) the difference spectrum showed a large negative peak at about 720 nm, a small negative peak at 650 nm, an isosbestic point at about 680 nm and a broad positive deflection from about 620 to 550 nm. Reversing the direction of the K^+ gradient (4 → 90 mM) inverted the difference spectrum about the zero axis. Spectra recorded under a variety of conditions including linearly polarized light (90° and 0° to the fiber axis) and the presence or absence of 50 µg/ml gramicidin had the same characteristic waveform. Difference spectra recorded from unstained fibers were flat. Supported by HHS HL 25418.

M-PM-Pos42 FUNCTIONAL DISRUPTION OF THE T-SYSTEM OF CUT MUSCLE FIBERS BATHED IN SOLUTIONS OF NORMAL TONICITY. Donald T. Campbell & Richard Hahin, Dept. Physiol. & Biophys., U. of Iowa, Iowa City, IA.

Single frog skeletal muscle fibers were studied using the Vaseline gap technique. The nodal pool contained normal Ringer; fiber ends were cut in isotonic CsF. The resistance in series with the surface membrane (R_s) was minimized by using separate current passing and voltage measuring electrodes in the nodal pool, and by placing a flap of plastic film on top of the Vaseline seals which by capillarity draws solution over the top surface of the fiber. R_s, estimated from the rapid initial "hop" in voltage resulting from a large step of current applied under current clamp conditions, was between 0.5 and 1.5 ohm-cm^2, values 10-25% of those determined using the original method (Hille & Campbell, 1976, J. Gen. Physiol. 67:265). Capacity transients elicited by 45 mV depolarizations from a prepulse of -135 mV show two distinct phases: a rapid phase due to charging of the surface capacitance (C_s) and a slow phase consistent with charging of the T-system (C_t). At the start of an experiment, the ratio of C_s:C_t is between 1:3 amd 1:8. Over 30-40 minutes this ratio declines to between 1:1 and 1:0.1 (see figure), suggesting that the T-system has become uncoupled from the surface. This uncoupling was seen both in shortened (1.2 um sarcomere spacing) and rest-length fibers. Treatment with Ringer made hypertonic with either glycerol or formamide produced a similar decline in 3-5 minutes, although fibers quickly deteriorated. When C_t:C_s is high and temperature is above 10°C, anomalous "bumps" in Na currents are frequently seen. After the decline of C_t, these anomalies are absent at all temperatures studied (up to 28°C). Supported by MDA and NIH (NS-15400).



M-PM-Pos43 SPREAD OF EXCITATION IN RAT CAUDAL ARTERY. Kent Hermsmeyer, Department of Pharmacology, The Cardiovascular Center, University of Iowa, Iowa City, Iowa.

Electrical interaction of vascular muscle cells in the rat caudal artery was measured using both intracellular (ic) and extracellular (ec) methods and compared to cardiac muscle. Using the same procedures, vascular muscle was shown to have less than 1/10 as much interaction as found in cardiac muscle, as follows. When spontaneous or electrically stimulated electrical spikes were recorded with ec pore electrodes, excitation spread over a strip > 10 mm in length in cardiac muscle compared to < 500 µm for vascular muscle (which represented the minimum measurable distance). In the 2nd method, strips of cardiac or vascular muscle were bathed in low viscosity silicone oil and electrical depolarization initiated at one end, according to the method of Weidmann (J. Physiol. 210:1041-1054, 1970). The maximum ratio of ec recorded depolarization over ic recorded depolarization was 0.18 in cardiac muscle and < 0.001 (the detection limit) in vascular muscle. No recording of depolarization spread in vascular muscle in silicone oil was successful in > 30 attempts, even though the same preparations showed norepinephrine depolarization and contraction. In the 3rd method, isolated vascular muscle cells from neonatal rat artery or vein were compared to ventricular myocardial cells. Synchronized contractions occurred over chains up to 48 cells long in cardiac muscle, but never more than 2 cells in vascular muscle. Thus, measurements by ec and ic recording and observation of isolated groups of 2-50 cells suggest that coupling in vascular muscle is dramatically less than that in cardiac muscle. In fact, these results are compatible with lack of electrical interaction between vascular muscle cells. The alternate hypothesis is that coordination of contraction in vascular muscle occurs by diffusion of norepinephrine; the measurements made in these studies would be compatible with such a hypothesis.

M-PM-Pos44 ELIMINATION OF CHARGE MOVEMENT IN SKELETAL MUSCLE BY A CALCIUM ANTAGONIST. C.S. Hui, R.L. Milton, and R.S. Eisenberg. Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907 and Dept. of Physiology, Rush Medical College, Chicago, IL 60612.

Charge movement was recorded from frog skeletal muscle fibers paralyzed by D-600 treatment at 7°C (McCarthy et al, these Abstracts). The voltage clamp methods and data analysis are those of Chandler et al, 1974 (J. Physiol. 254:245) and Gilly and Hui, 1980 (J. Physiol. 301:175). Nonlinear charge movement and contraction were observed in the presence of D-600, before the fiber was exposed to 190 mM K^+. After conditioning contractures, charge movement and contraction were abolished in the voltage range examined, -80 to +70 mV. Warming paralyzed fibers in the presence of 11.8 mM Ca^{++} re-establishes both charge movement and contraction.

These results suggest that during depolarization D-600 binds to a site involved in charge movement as well as excitation contraction coupling.

M-PM-Pos45 PARALYSIS OF SKELETAL MUSCLE FIBERS BY A CALCIUM ANTAGONIST. R.T. McCarthy, R.L. Milton, and R.S. Eisenberg. Department of Physiology, Rush Medical College, Chicago, IL 60612.

Single frog muscle fibers exposed to a 30 μM concentration of the calcium antagonist D-600 (methoxyverapamil) contract only once in response to a 190 mM K^+ solution at 7°C. After the first contracture, the fibers do not respond again to K^+, nor do they give twitches or tetani in response to electrical stimulation. These paralyzed fibers respond normally to caffeine, suggesting that their sarcoplasmic reticulum (SR) contains and is able to release normal amounts of calcium. The resting membrane potential and the action potential of paralyzed fibers are normal. Treatment in the warm (22°C) does not produce paralysis; furthermore, paralysis, produced in the cold, disappears if the fiber is warmed.

These results suggest that during depolarization D-600 binds to a site essential for excitation-contraction coupling. The anatomical location of the proposed site is unknown. D-600 might block a calcium channel in the SR membrane involved in calcium release (although the SR in paralyzed fibers releases Ca^{++} in response to caffeine); the drug might block a component of calcium current across the T membrane essential for T-SR coupling ('trigger' Ca^{++} hypothesis); the drug might immobilize a charge movement in the T membrane essential for T-SR coupling ('remote control' hypothesis); or the drug might block the flow of ionic current from T to SR ('electrical coupling' hypothesis).

M-PM-Pos46 A 'RESISTIVE BARRIER' AS A MEANS OF FACILITATING PROPAGATION THROUGH THE CARDIAC PURKINJE-VENTRICULAR JUNCTION. Richard D. Veenstra, R.W. Joyner, D.A. Rawling, and John Picone, Dept. of Physiology and Biophysics, The University of Iowa, Iowa City, Iowa, 52242.

At the Purkinje-Ventricular junction (PVJ) there are changes in action potential shape (e.g. $\dot{V}_{max}$ and duration), a conduction delay, and a low safety factor for propagation, as compared to the safety factor for propagation within the Purkinje strands or within the ventricular muscle. Previous workers have explained these results in terms of a 'functional discontinuity' at the PVJ or an anatomical 'funnel', coupling the large electrical load of the ventricular region onto the small Purkinje strand. We used both intracellular and extracellular recordings to identify the spatial extent and activation sequence of the PVJ of canine right anterior papillary muscles. The experimental results and our numerical simulations of action potential propagation through a model PVJ have led us to propose that there is a 'resistive barrier' produced by a relative lack of cell-cell coupling between Purkinje and ventricular cells. We propose that this 'resistive barrier' accounts for the observed conduction delay and actually facilitates action potential propagation at the PVJ by partially uncoupling the large ventricular region from the small Purkinje strand. We find that experimental interventions which tend to produce electrical uncoupling of cardiac cells (e.g. rapid stimulation rates, intracellular Ca^{2+} accumulation) increase the conduction delay through the PVJ with minimal effects of conduction velocity either within the Purkinje strands or within the ventricular muscle. The 'resistive barrier' hypothesis is also compatible with earlier histological studies showing 'transitional' cells in the PVJ region with few intercellular junctions.

M-PM-Pos47 RESIDUAL TENSION IN CAT INTRAFUSAL MUSCLE FIBERS. R.S. Wilkinson and C.C. Hunt, Dept. of Physiology and Biophysics, Washington Univ. School of Medicine, St. Louis, Mo. 63110.

Sensitivity of the primary ending of the mammalian muscle spindle to stretch is reduced following a large extension (Hunt and Wilkinson,J. Physiol. 1980, 302, 241) and enhanced after a period of fusimotor stimulation (Baumann, Emonet-Dénand and Hulliger, Brain Res. 1982,232, 460). Such changes in gain possibly result from changes in compliance of intrafusal fibers.

Isolated spindles have been used to study these changes. One spindle pole was attached to a tension transducer and stretch applied to the other; receptor potential was recorded from the primary ending. Receptor potential response to small amplitude sinusoidal stretch fell abruptly after a large stretch and recovered over a period of several sec. Following direct stimulation of the intrafusal bundle through Ag:AgCl wire electrodes, a residual increase in tension was observed. This was abolished by a large stretch; it appears related to the after-effects of fusimotor stimulation. In other experiments decapsulated isolated spindles were observed with Nomarski optics. Depolarizing current in the bag_1 fiber caused contraction followed by a smaller residual sarcomere shortening; this may also be related to the after-effects of fusimotor activity. Measurement of length changes with stretch shows that, for small stretches, intrafusal fibers are relatively more compliant in the sensory region than elsewhere. Modulation of compliance in the latter regions appears to be an important mechanism for gain regulation. (Supported by grants from the USPHS [NS 07907] and Muscular Dystrophy Association)

M-PM-Pos48 DIFFERENTIAL EFFECTS OF K-FREE RINGER AND OUABAIN ON INTRAFIBER K AND Na DISTRIBUTION IN FROG SKELETAL MUSCLE. C.N. Fong and J.A. Hinke, Department of Anatomy, University of Ottawa, Ottawa, Ontario K1H 8M5 Canada.

Intracellular sodium $(^aNa)_i$ and potassium $(^aK)_i$ activities were measured by ion-selective microelectrodes and total intrafiber sodium $[Na]_i$ and potassium $[K]_i$ were measured by flame analysis before and after the sartorius muscle was superfused for 40 min. by altered Ringer solutions. In K-free Ringer, $(^aK)_i$ fell by 36mM but $[K]_i$ remained unchanged; $(^aNa)_i$ increased by 16mM but $[Na]_i$ increased by only 5 mmole/kg f.w. In Na-free + K-free Ringer, both $(^aK)_i$ and $[K]_i$ remained unchanged whereas $(^aNa)_i$ and $[Na]_i$ decreased by 7mM and 9 mmole/kg f.w. respectively. After ouabain perfusion, $(^aK)_i$ fell by 27mM and $[K]_i$ fell by 12 mmole/kg f.w.; $(^aNa)_i$ increased by 7mM and $[Na]_i$ increased by 6 mmole/kg f.w.

None of these superfusions altered the fiber water content. Intrafiber pH (as measured by pH microelectrodes) remained constant during the superfusions. Interestingly, $(^aK)_i$ did not decrease in response to K-free Ringer in detubulated fibers. Thus, patent t-tubules and external Na seem essential before $(^aK)_i$ decreases. All observed changes can be explained by a two compartment model in which free K is in 20% of the intrafiber water. K-free Ringer induces free K to shift into the 80% compartment whereas ouabain permits K to exit the fiber from both compartments.

Supported by the Medical Research Council of Canada.

M-PM-Pos49 DETERMINATION OF IONIZED Mg CONCENTRATION IN SKELETAL MUSCLE FIBERS WITH MAGNESIUM SELECTIVE MICROELECTRODES. López, J.R., Alamo, L., Caputo,C., Vergara, J.*, DiPolo, R. Centro de Biofísica y Bioquímica, IVIC, Apartado 1827, Caracas, Venezuela, and Physiol. Dept. UCLA, Los Angeles, USA.

We have measured the intracellular free Mg^{2+} concentration in skeletal muscle fiber by using glass microelectrodes filled with Mg^{2+} selective neutral carrier (ETH 1117, kindly given by Prof. Simon). In the absence of interfering ions, they gave Nernstian responses between 1 and 10 mM free Mg^{2+}. However, in the presence of an ionic background resembling the myoplasmic composition, the microelectrode behavior was subnerstian (18-24 mV). The electrodes were calibrated before and after muscle impalements. In quiescent fibers from sartorius muscle (Rana pipiens),with resting membrane potentials not less than -81 mV, the intracellular free Mg^{2+} concentration was 3.8 ± 0.24 (SE)mM, n=48 at 21°C. In muscle fibers dissected from frogs adapted to cold (kept at 5°C for at least three weeks) the intracellular free Mg^{2+} concentration was 6.2 ± 0.45 (SE) mM, n=16. No significant change in the intracellular free Mg^{2+} was observed following extensive (≃ 6 hs) incubation in Mg^{2+} free media. Increasing the external concentration magnesium from 4 to 20 mM (≃ 15 min) produced a slow and small enhancement (1.8 mM) of $\{Mg^{2+}\}_i$, which was fully reverted when the divalent cation was removed from the bathing solution. No change in ionic magnesium resting concentration was observed when the muscle fibers were treated either with caffeine 3 mM or with Na^+ free solutions. Partially supported by CONICIT S1-1148.

M-PM-Pos50 EFFECTS OF ELEVATED EXTRACELLULAR CALCIUM ON EARLY AND LATE AFTERPOTENTIALS IN FROG SKELETAL MUSCLE. John N. Howell, Anuraj Shankar, and Tebogo Molefhe. Department of Zoological and Biomedical Sciences and the College of Osteopathic Medicine, Ohio University, Athens, OH 45701

The early afterpotential (EAP) consists of two portions, an initial notch and hump (N&H) and a subsequent exponential decay (ED) back to resting potential. It has been suggested that the N&H reflects the process of action potential conduction within the t-system whereas the ED portion, whose time constant closely matches that of the resting membrane, reflects the recharging of the membrane capacitance through the resting leak conductance after an action potential occurs. Raising extracellular calcium ion concentration from 1 to 10 mM, which causes t-tubular conduction to become decremental, greatly alters the shape of the EAP. The exponential phase begins earlier; it has a somewhat shorter half-time, and the N&H is eliminated. The shortening of the half-time suggests that either membrane resistance and/or effective capacitance during the EAP is lower in the presence of elevated extracellular calcium or that the ED reflects something other than the simple RC properties of the membrane. Elevated extracellular calcium also greatly suppresses late afterpotentials, which are thought to result from potassium accumulation in the t-system secondary to repetitive firing of action potentials within the tubules. The effects of elevated calcium are mimicked by like concentrations of spermine, a tetravalent cation at pH 7.2, but not by ruthenium red, a hexavalent cation, which probably does not gain entrance to the t-system. Ruthenium red affects the action potential in the same way as elevated calcium concentration does, but has little effect on either early or late afterpotentials. (Supported by NIH grant AM30132 and by funds from the Ohio University College of Osteopathic Medicine.)

M-PM-Pos51 LOCAL ACTIVATION IN INTACT, ISOLATED, TTX-TREATED RAT CARDIAC MUSCLE CELLS Barry London, Dept. of Physiology & Biophysics, The Albert Einstein College of Medicine, Bronx, N.Y. 10461

Localized electrical stimuli were used to evaluate one aspect of E-C coupling in single, unattached, intact, enzymatically isolated cardiac muscle cells. Extracellular micropipets (1-2 μm tip diam) were positioned adjacent to the cells to locally depolarize the cell membrane. Negative pulses (constant current, 2-10 μA, 2-50 msec) triggered uniform, all-or-none twitches, similar to those seen using distant electrodes (Krueger et al., J. Gen. Physiol. 76: 587, 1980). After the addition of 30 μM TTX, however, depolarizing pulses of similar magnitude and duration produced twitch-like shortening to sarcomere lengths as short as 1.6 μm, but which were radially symmetric about the pipet tip, and remained localized; the shortening did not propagate to distant parts of the cell. The local contractions rapidly and spontaneously relengthened, despite current pulses as long as 5 seconds. The area of shortening was gradable (involving 2-15 sarcomeres, with 1-10 μm inward spread), increasing with increased stimulus current (up to those currents which irreversibly damaged the cell), and with increased stimulus duration, but decreasing with increased stimulus frequency (to 5 Hz). Speed of relengthening was slowed by the addition of 10 mM caffeine. Localized shortening was reversibly inhibited by the addition of 50 μg/ml verapamil, by iontophoretically applied EGTA, and by 60 mM KCl.

These results demonstrate that in TTX-treated heart cells, a local depolarization (1) leads to a local increase in myoplasmic calcium which (2) is transient in nature. Moreover, (3) an amount of calcium sufficient to locally activate the myofilaments need not trigger the complete spread of activation throughout the intact heart cell. Supported, in part, by the NYHA and HL 21325-06.

M-PM-Pos52 TECHNIQUE TO DETERMINE THE SITE OF ORIGIN OF SLOW WAVES FROM A 2-DIMENSIONAL SHEET OF SMOOTH MUSCLE. N.G. Publicover, A.J. Bauer and K.M. Sanders. (Intro. by J. Peacock) Department of Physiology, University of Nevada, Reno, NV 89557.

The site of origin of spontaneously occurring slow waves (SW's) can be determined using a 2-step procedure by: (i) measuring the conduction velocity (CV) of evoked SW's in each dimension in the tissue, and (ii) measuring the time of arrival of spontaneous SW's from at least three recording sites. Rectangular sheets of canine muscle were dissected free of the stomach and maintained in Krebs solution. An extracellular electrode was used to elicit SW's at a known location in the tissue. Two recording electrodes were positioned in a line from the stimulating electrode parallel to the circular muscle fibers. Intracellular micropipettes or extracellular surface electrodes can be used to measure the time of arrival of induced SW's at each location. The CV was determined by dividing the distance between the recording electrodes by the average difference in the time of arrival of SW's at the two sites. The process was repeated in the perpendicular direction.

Recordings during spontaneously activity were then made simultaneously from 3 sites. A binary search was performed by computer to locate the position on the tissue which results in the measured differences in the time of arrival of the SW at each recording site. For best results, electrodes were placed as far apart as possible (preferably in three corners of the tissue). Spontaneous activity was monitored for periods up to an hour to identify dominant regions of SW origin. This technique can be used to study agents or conditions which can change the origin and the resultant direction of propagation of electrical events in muscle tissues. (Supported by the American Heart Association and NIH AM32176.)

M-PM-Pos53 LOW FREQUENCY IMPEDANCE OF FROG MUSCLE FIBERS. R.F. Valdiosera, E. Ramirez, and B. Mendiola. Depto. Farmacologia, CINVESTAV I.P.N., Mexico.

The impedance of frog skeletal muscle fibers was measured in the frequency range of 0.2 to 10^4 Hz in normal Ringer solution. The data above 1 Hz agree with other workers, however, below 1 Hz, a new 4-8 degree dispersion with a f_o of about 0.5 Hz was found. This dispersion may be represented by a series combination of a capacitor (50-70 $\mu F/cm^2$) and a resistor (8-10 $k\Omega$-cm^2) that would add in parallel to the known electrical properties of muscle cell membranes. Several possibilities have been explored in order to explain this finding. It could be caused by accumulation or depletion of permeant ions in the tubular system. It has been shown theoretically that differences in transport numbers in the lumen and the wall of the tubular system give rise to a large capacitance and therefore this type of effect seems to be critical for the interpretation of impedance measurements below 1 Hz. To minimize this effect, the K^+ concentration in the Ringer was raised to 7.5 mM with very little if any effect on the dispersion. Also, Rb^+ was substituted for K^+ to avoid nonlinear behavior of the inward rectifier, and in this case the dispersion seemed to be smaller with low concentrations of Rb^+ (0.5 mM) than with higher concentrations (15 mM). This dispersion could also be explained by an as yet undiscovered pathway for current flow across the cell membrane. A particularly interesting possibility would be current flow between the tubular system and the sarcoplasmic reticulum. The value found for the low frequency capacity (50-70 $\mu F/cm^2$) is close to the expected membrane capacity of the sarcoplasmic reticulum.

M-PM-Pos54 AN INVESTIGATION OF DYE-SENSITIZED BILAYER LIPID MEMBRANES

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Dyes have been used for well over 150 years for bacteriology, cytology, hematology and histology. More recently, dyes have also been used as sensitizers in both inorganic and organic semiconductors for the purpose of understanding the mechanisms of photoeffects and also for the practical application of photosensitive materials. Two types of sensitization are known: intrinsic and spectral. In the former, the added sensitizer does not alter the basic spectral response of the system, but merely enhances it. In spectral sensitization, the system is modified by the sensitizer in the region of the spectrum corresponding to the optical absorption of the added sensitizer (See BLM: Theory and Practice, Dekker Inc., 1974, p. 297). Owing to the importance of dyes, we have initiated a project to investigate systematically dye-sensitized bilayer lipid membranes (BLM) using the cyclic voltammetry technique. 57 dyes of different chemical groups have thus been investigated. From the obtained voltammo-grams, 5 types of characteristic curves have been established. Electronic processes in as well as across the BLM are considered. Redox reactions at the BLM/solution interfaces are most conveniently studied by this technique. In the presence of light of known wavelengths, a number of dye-sensitized BLMs have also been investigated by photoelectrospectrometry (BBA, 597, 433-444, 1980), the details of which will be presented.

(This work was supported by PHS GM-14971)

M-PM-Pos55 SMALL ANGLE X-RAY SCATTERING (SAXRS) OF DIMYRISTOYL LECITHIN (DML)/CHOLESTEROL OLEATE (CO) MICROEMULSIONS:STRUCTURAL ANALOGUES OF LOW DENSITY LIPOPROTEIN (LDL). G.S. Ginsburg and D. Atkinson, Biophysics Inst.,Boston Univ. Schl. Med., Boston, Mass.

As models for the lipid organization of LDL, DML/CO microemulsions are stable particles with chemical composition (CO/DML=0.9 m/m), Stokes radius (94±2Å)and diameter from electron microscopy (198±19A)consistent with a spherical particle with a CO core, stabilized by a surface monolayer of DML. At 4°C, the solvent electron density (contrast) dependent SAXRS profiles [I(h)] show well defined subsidiary maxima indicating a homogeneous population of quasi-spherical particles. The contrast independent absolute intensity and the temperature dependent relative intensity of the maximum at $1/36Å^{-1}$, and a wide angle diffraction at $1/5Å^{-1}$ indicate an ordered liquid crystalline packing of the CO in the particle core. A wide angle diffraction at $1/4.2Å^{-1}$ demonstrates an ordered packing of the DML fatty acyl chains in the surface monolayer. The mean particle electron density ($\bar{\rho}$=0.335 e/Å^3) determined from the contrast dependent $I^{\frac{1}{2}}(0)$ agrees with the chemical composition. The radius of gyration of the particle shape ($R_g \approx 78Å$) agrees with the particle radius (100A) from independent estimates. Model building analysis assuming a radial step function electron density distribution shows the experimental I(h), distance distribution functions from the Fourier Transform (FT) of I(h), and the radial electron density distribution from FT of $I^{\frac{1}{2}}(h)$ to be consistent with a spherical model containing 6 electron density regions:ρ_1=0.28, R_1=8Å;ρ_2=0.42, R_2=16Å;ρ_3=0.29, R_3=44Å;ρ_4=0.37, R_4=56Å;ρ_5=0.29, R_5=87Å;ρ_6=0.40, R_6=97Å. As structural models for LDL lipids, DML/CO microemulsions at 4°C show that (i) the core located CO is packed in a disordered smectic phase, and (ii) the surface located polar shell is 10Å thick vs. 25Å in LDL suggesting a large protein contribution to the surface shell of LDL.

M-PM-Pos56 EFFECTS OF HEXANE ON LIPID BILAYER STRUCTURE. Glen King and Stephen White, Dept. of Physiology and Biophysics, University of California, Irvine, CA. 92717

We have examined the effects of hexane on the structure of oriented dioleyl lecithin (DOL) bilayers to understand better the interactions of small hydrophobic molecules with lipid bilayers. Neutron diffraction methods can be used to measure the amount of hexane in the bilayer and its distribution with respect to thickness. We reported earlier[1] that hexane at low concentrations is located largely in a zone 10A wide at the center of the bilayer. We have now extended our studies to include high hexane concentrations. A significant finding is that up to 0.84 hexane molecules per acyl chain enter the hydrocarbon region of the bilayer without changing the volume of the region. Thus, the hexane appears to fill in "voids" of some sort that already existed in the bilayer. However, before significant amounts of hexane can enter the bilayer, it undergoes a "transition" which apparently allows a consolidation or "chunking" of the free volume to an extent that it can accomodate the hexane molecules. Another result of this "transition" is that relatively more hexane can enter the hydrocarbon region adjacent to the headgroups, although the majority is still located in the central region. From our neutron scattering density profiles on an absolute scale, we also find that the density of packing in the hydrocarbon region of bilayers without hexane is somewhat less than in the corresponding bulk alkane (9-octadecene). A further finding is that the area per molecule in hexane free bilayers is about 74A^2, which, combined with lipid width d_1 from strip function models, results in a calculated lipid mass density of 0.8-0.9 gm/ml.

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M-PM-Pos57 ALTERNATING CURRENT STUDIES OF VALINOMYCIN-MEDIATED ION TRANSPORT ACROSS LIPID BILAYERS
A.D. Pickar and K.L. Cox, Portland State University, Portland, Oregon 97207.

Alternating current admittance measurements were used to study the kinetics of the valinomycin-Na^+ and valinomycin-Rb^+ transport systems in a series of glycerolmonooleate membranes. Measurements which yield reliable values of membrane conductance and capacitance over the frequency range 0.2 - 200 kHz can be obtained within 45 seconds using an automatic balancing bridge under the control of a microprocessor unit. The measured frequency dependence of conductance was compared with the theoretical expressions derived by Kolb and Läuger [1] using a curve fitting procedure which yields values for both interfacial complexation and translocation rate constants. The frequency dependence of capacitance gives rate constants which are consistent with but not as reliable as those obtained from conductance information. Combined with auxiliary measurements of direct current conductance, surface densities of carrier complexes are also obtained. Values of rate constants derived from alternating current measurements agree in most cases with those obtained from charge pulse experiments by Benz and Läuger [2] and also show similar trends with changes in experimental conditions. Discrepancies are most serious for cases of low metal ion concentrations and for the loaded carrier translocation rate constant. There is evidence that the difference between the alternating current and relaxation results is related to non-specific dielectric losses occurring in our membranes. (Supported by NIH Grant 5R01 ES937-08.)

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[2] Benz, R., and Läuger, P., J. Memb. Biol. 27 171 (1976).

M-PM-Pos58 HEAVY METAL (THALLIUM, CADMIUM AND MERCURY) TRANSPORT THROUGH LIPID BILAYER MEMBRANES.
John Gutknecht, Dept. of Physiology, Duke University, and Duke Marine Lab., Beaufort, N.C. 28516.

Heavy metal transport is important in physiology and toxicology, but the mechanisms of transport are largely unknown. We studied the diffusion of inorganic Tl^+, Cd^{2+} and Hg^{2+} through planar lipid bilayer membranes formed from diphytanoylphosphatidylcholine in decane. Tracer fluxes (^{204}Tl, ^{109}Cd and ^{203}Hg) and electrical conductances were measured, and the permeabilities to nonionic and ionic forms of the metals were calculated. The conductive (ionic) permeability of all the metals was low. The permeability to Tl^+ was 10^{-11} cm/sec, which does not support previously published reports that Tl^+ is a "lipid permeable" cation in biological membranes and liposomes. The tracer fluxes of all metals were dependent upon the Cl^- concentration but not pH. From the tracer fluxes, the unstirred layer thickness, and the association constants for metal-Cl complex formation, we estimated the membrane permeabilities to the nonionic forms, i.e., TlCl, $CdCl_2$ and $HgCl_2$. Permeabilities ranged from about 10^{-5} cm/sec for TlCl to 10^{-2} cm/sec for $HgCl_2$. These high permeabilities suggest that the biological uptake of heavy metals may occur by diffusion of electroneutral Cl complexes. The ability of Tl^+ to behave as a lipid permeable cation in biological membranes can also be explained by the diffusion of TlCl or $TlNO_3$. Theoretical calculations predict identical intracellular concentrations of Tl regardless of whether Tl crosses the membrane as Tl^+, TlCl or $TlNO_3$, provided that Cl^- and NO_3^- are in equilibrium across the membrane. (Supported by USPHS grants ES 02289 and GM 28844.)

M-PM-Pos59 CHANNEL VERSUS CARRIER IONOPHORE ACTIVITY IN LIPID VESICLES DIFFERENTIATED WITH A VOLTAGE SENSITIVE PROBE.
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We have developed an assay for ionophoric activity which can differentiate channel-forming and carrier behavior. It can also be used to distinguish between multi- and unilamellar phospholipid vesicles. This is accomplished by following the collapse of a valinomycin mediated K^+-diffusion potential across the vesicle membrane using a potential-sensitive fluorescent dye. Experiments performed with gramicidin, a known channel former, on multilamellar vesicles showed a slow decay of the potential which was only slightly dependent on the channel concentration; unilamellar vesicles, on the other hand, demonstrated an instantaneous dissipation of the potential. This distinction may prove useful in differentiating between these types of vesicles. With well-characterized unilamellar vesicle preparations, the rates of channel transfer and carrier-mediated flux are obtainable. In each vesicle system the limit on the rate data imposed by the kinetics of fluorescent probe redistribution is determined by stopped-flow fluorometry. The experiments with gramicidin can all be interpreted in terms of a model in which the channel mediates instantaneous collapse of ion concentration gradients but with observed kinetics that may be limited by the rate of gramicidin transfer from one bilayer to another. For carriers like monensin, the rate of bilayer-bilayer transfer can be faster than the ionophore mediate ion-flux. This contrasting behavior for channel and carrier ionophores should be general.

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M-PM-Pos60 STEADY STATE EQUATIONS FOR MEMBRANE POTENTIAL IN TERMS OF JK, JNa, GK, GNa, (K)o and (Na)o. Francis G. Martin, Division of Biophysics, Department of Physiology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140.

In an effort to understand the relationship between the activity of the sodium-potassium pump and the steady state ionic distributions which result from that activity, an equivalent electrical circuit is used to conceptualize some of the constraints placed on a system where current is carried by more than one ionic species. In a hypothetical system consisting of two aqueous solutions (inside and outside) separated by a membrane, the current carriers are sodium and potassium since the membrane is permeable to these two ions. Chloride is present on both sides of the membrane to maintain electroneutrality but the membrane is not permeable to it. The steady state of the system is defined as that state in which there is no change in the distribution of ionic concentrations in either compartment with time. The individual ionic components of the pump current (JK & JNa), the specific ionic conductances of the membrane (GK & GNa), and the ionic concentrations in the outside aqueous solution ((K)o & (Na)o) are considered to be independent variables. The membrane potential (Em), the Nernst potentials for potassium and sodium (EK & ENa), and hence the ionic concentrations in the inside solution ((K)i & (Na)i) are the unknown dependent variables. Consideration of the system as described indicates that the potential represented by the difference between EK and ENa is of such a value so as to prevent the flow of current between the two ionic systems.

M-PM-Pos61 PHOTO-ELECTRIC EFFECTS IN MEMBRANES AS DETECTED WITH ELECTROMETERS AND VOLTAGE CLAMPS J. S. Huebner, R. T. Arrieta & I. C. Arrieta, University of North Florida, Jacksonville, FL 32216

A comparison of laser flash induced photo-effects in bilayer membranes detected with electrometers and voltage clamps is presented, using photo-effects induced by dyes previously studied[1-4]. The electrometer and voltage clamp used 2 GHz operational amplifiers. Experiments with equivalent circuits and current function generators, as well as membranes and dyes, demonstrated that signals from both apparatus depend on cell geometry, aqueous solution salt concentrations and electrode resistance. Under favorable circumstances, electrometers followed the membrane voltage with unity gain from DC to 75 MHz. Voltage clamps provided signal gains of typically 500, but only below $\sim$ 15 kHz. This frequency dependence makes flash induced voltage clamp signals complex functions of the charge displacement sequences inducing the photo-effects. Quantitative expressions derived for voltage clamp signals for simple cases agreed with experimental results obtained using both equivalent circuits and current function generators, and membranes, dyes and 10 nsec laser flashes. These results will be described; they demonstrate voltage clamp signals resulting from light flashes are not simply related to the membrane photo-current or to the net quantity of charge transported, as has been repeatedly claimed. Supported by NIH grant GM 23250.

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4. J. S. Huebner, R. T. Arrieta and I. C. Arrieta, submitted

M-PM-Pos62 INVESTIGATIONS OF THE INTERACTION OF ALPHA PARTICLES WITH PLANAR LIPID BILAYERS. Gene A. Nelson, Dept. of Biophysics, 118 Cary, SUNYAB , 3435 Main St., Buffalo, NY 14214

Understanding the nature of the interaction of alpha particles with planar lipid bilayers is important in the areas of nongenetic radiation damage studies and in pi-meson or heavy ion cancer therapy. However, the range of 5 MeV alpha particles from Polonium-210 in aqueous media is only approximately 60 microns. This short range makes any effort to study the system difficult. A system utilizing a compact voltage clamp and a mechanical advancing scheme for a solid source will be described. Membrane stability is reduced by the Polonium source. Audio spectrum analysis and FFT analysis show an alteration of the current noise spectrum of the bilayer after it is exposed to Polonium. Evidence seems to favor a free-radical mediated alteration of the bilayer structure.
Research supported in part by the Biophysics Company, Amherst,NY

M-PM-Pos63 THE ROLE OF GTPase ADENYLATE CYCLASE IN MODULATING THE SODIUM EFFLUX IN BARNACLE MUSCLE FIBERS. E.E. Bittar and J. Nwoga, Dept. of Physiology, Univ. of WI, Madison, WI 53706.

It is now widely recognized that cholera toxin (and its subunit A fragment) causes in cells irreversible activation of adenylate cyclase as the result of inhibiting GTPase activity by ADP-ribosylation. Cholera toxin (CT) in a concentration as low as 10^{-7}M, when injected into ouabain-poisoned barnacle muscle fibers causes sustained stimulation of the Na efflux i.e. an effect which mimics that seen following the injection of pure cAMP-protein kinase type 2 catalytic subunit (J. Physiol. Dec. issue 1982). However, the onset of stimulation is always preceded by a lag phase (15 ± 1 min, n=17) and is rather slow to reach a maximum (66 ± 5 min, n=17). Enrichment of these fibers with NAD^+ fails to enhance the response to CT. In general, prior injection of GTP or Gpp(NH)p markedly reduces the response to CT; prior injection of CT reduces the response to guanine nucleotides. Sudden omission of external Ca^{2+} following peak stimulation by CT leads to a large fall in the Na efflux which is almost completely reversed by restoring external Ca^{2+}. Injection of EGTA before or after CT reduces the response. That myoplasmic pCa falls following CT injection is indicated by the presence of an increased glow with aequorin. The reduced pCa is unlikely to be due to Ca^{2+}-channel activation, since the response to CT is unaffected by prior application externally of verapamil or Cd. Injection of PKI, Mg, Zn and Fe, or external applications of imipramine, chlorpromazine and mepacrine have a marked inhibitory effect on the response to injected CT. Collectively, these experiments support the idea that sustained stimulation of the ouabain-insensitive Na efflux is the result of persistent activation of adenylate cyclase by the toxin and that a fall in internal pCa enhances this activation mechanism, possibly through the involvement of calmodulin.

M-PM-Pos64 AMILORIDE-SENSITIVE Na^+ INFLUX IN CULTURED C6 GLIOMA CELLS. D.J. Benos, V.S. Sapirstein, and E.W. Overstrom, Departments of Physiology and Biophysics, and Biological Chemistry, Harvard Medical School, Boston, MA 02115.

We have studied the induction of an amiloride sensitive, Na entry mechanism into cultured rat C6 glioma cells. Cells grown continuously in the presence of 10% fetal calf serum (FCS) had amiloride-insensitive Na influxes of 20-25 nmoles/mg protein min. Removal of serum for 24 hr resulted in a slight decrease in total Na influx. However, readdition of serum to the incubation medium caused a 50-100% increase in Na influx within 2 minutes. This increased influx was inhibited by amiloride (K_D = 0.4 mM). At least 4 hrs of serum deprivation was required to observe a detectable increase in amiloride sensitive influx. Addition of cycloheximide (5 µg/ml) or fenfluramine (0.1 mM) blocked the serum induction of this transport system, suggesting that a coordinated synthesis of lipid and protein is required for expression. We have also found that this serum stimulated Na influx did not saturate with $[Na_o]$, and that only amiloride was capable of inhibiting this transport system. Plasma membrane protein synthetic patterns of C6 cells before, during and after the induction of this Na entry system was studied using 1 and 2 dimensional gel electrophoresis techniques. Preliminary results suggest the insertion of a 90-100k dalton protein into the plasma membrane of quiescent C6 cells within 2 minutes of serum reintroduction. Likewise, thin section transmission electron microscopy suggests high rates of membrane turnover activity, with possible fusion of submembrane vesicles with the surface membranes. These results support the hypothesis that during serum deprivation C6 cells synthesize a Na^+ transport protein which is functionally recruited from a sub-plasma membrane population of vesicles. Supported by NIH Grants AM 25886, HD05515, and NS16186.

M-PM-Pos65 IDENTIFICATION OF THE GLUCOSE TRANSPORTER OF SKELETAL MUSCLE MEMBRANES BY PHOTOLABELING WITH ^{3}H-CYTOCHALASIN B (^{3}H-CB). Amira Klip and Denise Walker, Div. Neurology, The Hospital for Sick Children, 555 University Ave., Toronto, Ont. M5G 1X8, Canada.

The glucose transport system of muscle has been detected in isolated membranes of rat skeletal muscle by equilibrium binding of ^{3}H-CB (*Klip A and Walker D, Arch. Biochem. Biophys. in press 1982*). Identification of this transporter was attempted through covalent photolabeling with ^{3}H-CB. Effective labeling of plasma membranes was achieved by successive additions (5 x 0.05 µM) of ^{3}H-CB at 5 min intervals during irradiation at 280 nm with a Xenon arc. This was followed by polyacrylamide gel electrophoresis and determination of covalently bound radioactivity. Specific labeling (protected by 200 mM D-glucose) occurred only in one peak (D-glucose sensitivity 70%). The apparent Mr of this component varied in different gel systems: 38,000 in polyacrylamide gradients (5 to 15%) with glycerol, and 45,000 in 10% Laemmli gels. Between 1% and 20% of the total transporters were labeled and the rest still showed equilibrium binding of ^{3}H-CB. In contrast, such binding was lost when irradiation was performed with unfiltered light. pH-Denatured samples did not show D-glucose protectable photolabeling. In addition to the specifically labeled peak, non-protectable labeling occurred on the main polypeptide of these membranes (Mr 100,000). With mild trypsinization - which resulted in loss of the main polypeptide but did not affect the equilibrium binding of ^{3}H-CB - the label in the 100,000 region disappeared without affecting the D-glucose protectable peak. In summary, the Mr of the glucose transporter of muscle is similar to that recently determined in adipocytes, erythrocytes and fibroblasts. Moreover, conditions of photolabeling can be implemented that result in labeling of up to 20% of the transporter polypeptides. *Supported by the Medical Research Council of Canada.*

M-PM-Pos66 VOLTAGE DEPENDENCE OF AMILORIDE INHIBITION OF APICAL MEMBRANE Na CONDUCTANCE IN TOAD URINARY BLADDER. Lawrence G. Palmer, Department of Physiology, Cornell University Medical College, New York, N. Y. 10021

Inhibition by amiloride of apical membrane Na conductance of the toad urinary bladder was voltage dependent. Bladders were bathed with a high K-sucrose serosal medium to reduce series basal-lateral resistance. Transepithelial current-voltage relationships were measured during a time interval of one second over a voltage range of ±200 mV. Na channel I-V relationships were obtained by subtraction of currents measured in the presence of a maximal dose of amiloride (20 μM). With submaximal doses of amiloride, the degree of inhibition of Na channel slope conductance (G_{Na}) increased as the mucosal potential was made more positive. With 100 nM amiloride, G_{Na} was reduced by 29±4% at a transepithelial voltage of zero, and by 45±4% at 150 mV, mucosa positive. G_{Na} was reduced in a similarly voltage-dependent manner by mucosal H^+ (1 mM) and by mucosal K^+ (30 - 90 mM). These findings suggest that a cation binding site controls access to the channel from the mucosal solution. The site senses 15-20% of the transmembrane electric field, has an apparent pK_a of less than 3 at zero potential and is less ion-selective than the channel itself. (Supported by USPHS Grant AM27847 and the Whitaker Foundation).

M-PM-Pos67 DIFFUSION WITH SUPERIMPOSED CONVECTIVE SOLUTE TRANSPORT. F.E. Curry (Introduced by V.H. Huxley), University of California at Davis, CA 95616.

The relation for flux of solute, Js, in the presence of an external field which imparts a velocity, U_s, to the solute is: $Js = D_m\, dC/dx + U_sC$. C is the solute concentration and D_m the local diffusion coefficient. With a potential for water flow, $U_s = \chi U_w$ where χ is the velocity of the solute relative to the mean water velocity U_w. Integration across the membrane of thickness Δx with fluid flow from side 1 (high C) to side 2 yields:

$$Js = D_m/\Delta x\,(C_1 - C_2)\,[Pe/(\exp^{Pe}-1)] + U_w\chi C_1. \qquad (1)$$

Pe is the Peclet No. equal to $(U_w\chi\Delta x/D_m)$. Eqn. 1 enables the partitioning of solute flux into a convective component entering the membrane ($U_w\chi C_1$) and a diffusive component entering the membrane. The term $Pe/(\exp^{Pe}-1)$ is the solute concentration gradient at the membrane entrance $(dC/dx)_0$ expressed relative to the solute gradient in the absence of water flow, $(C_1-C_2)/\Delta x$. At high Pe, $[Pe/(\exp^{Pe}-1)] \approx 0$, and $Js = U_w\chi C_1$. For $Pe < 1$, $Pe/(\exp^{Pe}-1) \approx 1-Pe/2$, and the diffusive term is approximated by $(D_m/\Delta x)(C_1-C_2)(1-Pe/2)$. Rearrangement of Eqn. 1 yields:

$$Js = (D_m/\Delta x)(C_1 - C_2) + U_w\chi(C_1 + C_2)/2.$$

Confusion has arisen because $U_w\chi(C_1+C_2)/2$ has been called the convective component of flux in the membrane. This interpretation is incorrect. The convective component entering the membrane is always $U_w\chi C_1$. The term $(D_m/\Delta x)(C_1-C_2)$ overestimates diffusion. The diffusive component entering the membrane is reduced by the fraction $(1-Pe/2)$. Exactly the same arguments apply to the flux equation derived from the integration of the local thermodynamic equations. Supported by HL18010.

M-PM-Pos68 EFFECT OF INSULIN ON THE ELECTRICAL AND ION PERMEABILITY PROPERTIES OF THE AMPHIBIAN OOCYTE PLASMA MEMBRANE. Gene A. Morrill, Steven P. Weinstein, and Adele B. Kostellow. Department of Physiology and Biophysics, Albert Einstein College of Medicine, New York, New York 10461.

Insulin (0.1-10 μM) produces a negative-going hyperpolarization of the plasma membrane of the isolated prophase Rana oocyte and can initiate the meiotic divisions. Hyperpolarization begins within 5-15 min and is maximal (12-16 mV) by 30-60 min. The time course of hyperpolarization coincides with increased ^{22}Na efflux and with a decrease in ^{22}Na influx. As reported elsewhere (J. Memb. Biol. 69:41, 1982), the membrane potential of the prophase oocyte has a significant electrogenic component with potential but not conductance sensitive to ouabain. At maximal insulin-induced hyperpolarization, membrane conductance is decreased by one-half; neither membrane potential nor conductance are affected by ouabain. [^{3}H]-Ouabain binding by the plasma membrane of the untreated oocyte shows at least two components: high affinity sites ($K_d = 4.2 \times 10^{-8}$ M; 1000 sites per μm^2) linked to inhibition of the Na-pump and one or more classes of low affinity, high capacity sites. In contrast, insulin-treated oocytes show a single class of sites ($K_d = 1.4 \times 10^{-7}$ M) which are 2-3 times the number of high affinity sites found for the untreated oocyte. [^{125}I]-Insulin uptake by the plasma membrane is temperature-dependent and is maximal in less than 15 min at 20° C. Insulin binding to the plasma membrane shows at least two components: high affinity sites estimated to have a $K_d = 9 \times 10^{-8}$ M and 10-15 sites/μm^2, and one or more classes of low affinity, high capacity sites. These results suggest that insulin stimulates the Na-pump with $[Na]_i$ becoming rate-limiting at maximal hyperpolarization. The change in ouabain binding may reflect a decrease in Na-dependent phosphorylation of the Na, K-ATPase. (Supported in part by research grant HD-10463).

M-PM-Pos69 STEROL TRANSBILAYER MOVEMENT IN *MYCOPLASMA GALLISEPTICUM* MEMBRANES: EFFECTS OF CHOLESTEROL LEVEL AND SIDE CHAIN MODIFICATION. Sanda Clejan and Robert Bittman, Department of Chemistry, Queens College of The City University of New York, Flushing, NY 11367.

Mycoplasma are prokaryotes that lack cell walls and require cholesterol (Ch) for growth. The kinetics of Ch exchange between [^{14}C]Ch-labeled *M. gallisepticum* cells and an excess of small, sonicated egg phosphatidylcholine (PC)/Ch vesicles was measured as a function of Ch/phospholipid (PL) ratio at 37°C. The Ch level of *M. gallisepticum* cells was varied over a wide range by adaptation to growth in media containing 10 μg/mL each of oleic and palmitic acids and 2-10 μg/mL of Ch. The vesicles contained the same Ch/PL ratio. No net transfer was detected during the 14-h exchange period. The kinetics of exchange in sucrose-Tris-Mg^{2+} buffer, pH 7.8, with 2% albumin was biphasic. Both the fast and slow rates were enhanced as the Ch/PL ratio decreased. The half-time for equilibration, $t_{1/2}$, of the two Ch pools decreased from 6.2±0.3 h (Ch/PL=0.92); 5.5±0.5 h (Ch/PL=0.50); 4.6±0.5 h (Ch/PL=0.36); to 2.5±0.6 h (Ch/PL=0.25). The pool sizes of Ch were not affected by variation in Ch level. The exchange kinetics did not change markedly after extensive protein crosslinking with dimethylsuberimidate and N,N'-*p*-phenylenedimaleimide. These studies suggest that the transbilayer movement of Ch in *M. gallisepticum* membranes and the rate of Ch exchange to vesicles are controlled by the Ch level of these membranes. Cells of *M. gallisepticum* were also grown in media containing [^{14}C]-β-sitosterol, which has a 24α-methyl group. The exchange kinetics with an excess of β-sitosterol/PC vesicles (molar ratio of 0.90) was biphasic; $t_{1/2}$ was 7.4±0.6 h at 37°C. Three-quarters of the β-sitosterol was localized in the outer leaflet of the membrane. In contrast, [^{14}C] Ch was distributed symmetrically. Thus, the nature of the sterol side chain affects the extent and rate of sterol translocation. (Supported by NIH HL 16660 and Amer. Heart Assoc.-Nassau Chapt.)

M-PM-Pos70 LASER-EXCITED EU (III) SPECTROSCOPY USED TO CHARACTERIZE METAL ION BINDING TO CALCIPHORIN, A POSSIBLE CA (II) IONOPHORE. Tom R. Herrmann, (Eastern Oregon State College, La Grande, OR 97850), A. Ranjith Jayaweera, and Adil E. Shamoo, (University of Maryland School of Medicine, 660 W. Redwood St. Baltimore, MD 21201).

A low molecular weight protein named calciphorin has been isolated from calf heart, beef heart, and rat liver inner mitochondrial membrane. It appears to fulfil the criteria for a calcium carrier (Jeng et al, Proc. Nat. Acad. Sci. USA, 75: 2125-2129 (1978)). However, there has been some question as to whether the ionophoric characteristics reported are due to contaminants such as phospholipids or detergents. We have applied the recently developed technique of laser-induced Eu (III) luminescence to characterize Eu (III) binding to our calciphorin preparations and to possible contaminants. In particular, we have extensively characterized Eu (III) binding to a variety of phospholipids and to deoxycholate and digitonin. We are able to clearly differentiate Eu (III) binding in calciphorin preparations from binding to phospholipids (including cardiolipin) or detergents. We present this as evidence that previously reported Ca (II)-ionophoric activity of calciphorin is not due to contaminants. We will present data on hydration of the bound ion and on interpretation of excitation spectra. Supported by a Program Project of the N.I.E.H.S. (ES-1248), the Dept. of Energy (DEAS0580EV10329), the Office of Naval Res. (N00014-80-C-0030), and the Muscular Dystrophy Assoc. of America.

M-PM-Pos71 H^+ EFFLUX AND ACROSOME REACTION IN SEA URCHIN SPERM SUSPENDED IN Na^+ FREE SEA WATER. J. García-Soto and A. Darszon, Dept. Biochem. CINVESTAV-IPN México City.

The acrosome reaction in sea urchin sperm is induced by a glycoprotein "jelly" surrounding the egg, and requires Ca^{++} and Na^+ in the external medium at pH 8.0. During this reaction the intracellular levels of Ca^{++} and Na^+ increase and there is an efflux of H^+ and K^+. The sequence of these events and their relationship has not been established. pH values above 8.5 in sea water induce the acrosome reaction in the absence of jelly (Collins *et al*, Exp. Cell Res.106:211) while drugs such as D600 inhibit the transport of Ca^{++}, Na^+, H^+ and the acrosome reaction triggered by jelly (Shackman *et al*, Dev.Biol.65:483). We have observed that 84% of *Litechinus pictus* sperm undergo the acrosome reaction at pH 9.0 in Na^+ free (choline substituted) sea water (NaFSW) in the absence of jelly, this value being similar to the control (78%) which had the normal (Na^+). Addition of D600 at this pH decreased significantly the % of acrosome reaction in the presence or absence of Na^+. Also, it was found that H^+ release by sperm increased as the pH of NaFSW increased, and that this release was inhibited by D600. The results show a correlation between the % of acrosome reaction and the magnitude of H^+ release associated with this event. The fact that the acrosome reaction occurs in NaFSW when the pH is increased suggests that the change in Na^+ permeability associated with the jelly induced acrosome reaction could have a role only in the initiation of this event but that it is not required in later stages. (Supported by CONACYT and NSF).

M-PM-Pos72 POTASSIUM LOSS IN RED CELLS INDUCED BY EXTERNAL CALCIUM. J.S. Adorante and R.I. Macey. Dept. of Physiology-Anatomy, Univ. of Calif., Berkeley, CA 94720.

Human red cells pre-treated with low ionic strength solutions and resuspended in saline responded in a biphasic manner to extracellular Ca. Addition of Ca causes a large transient K^+ efflux of as much as 600 mM/liter cell H_2O/hr, followed by a decrease below control levels. The first pahse of this response (Phase I) resembled the Gardos effect in several respects. It is inhibited by oligomycin, external K^+ and by increased exposure time to Ca^{++}. Further, the P_K of Phase I is similar to the P_K of the Gardos effect ($5x10^{-8}$-$9x10^{-8}$ cm/sec) and the cells hyperpolarize in a low K^+ medium when Ca^{++} is added.

Differences between the Gardos response and Phase I of lactose-treated cells are: 1) La, which prevents the Gardos response, is ineffective on Phase I. 2) The pH maximum of Phase I is 6.8 while the Gardos response increases with increasing pH. 3) External Ba^{++} prevents the development of Phase I but not the Gardos response, while internal Ba^{++} prevents the Gardos response. 4) Attempts to demonstrate a Ca leak or pump failure during Phase I have failed; passive Ca^{++} movement of both treated and normal cells are similar. These results suggest that low ionic strength exposes Ca^{++} sensitive sites to the external medium. (Supported by NIH Grant GM18819)

M-PM-Pos73 **ANION ASSOCIATED PROTON FLUXES IN HUMAN RED BLOOD CELLS.** **Steen Dissing and Joseph F. Hoffman , Dept. of Physiol. Yale University, School of Med. New Haven, Ct 06510.**

We measured with a pH-stat the influx of protons into human red blood cells preequilibrated in media consisting of Cl,Br,I or SO_4 and 0.1 mM DIDS. The influx of protons was measured as a function of the outside proton concentration and the relative magnitude depends upon the anion present. When the outside pH is lowered to 6.50 in a 150 mM CholineCl medium (pH_i = 7.40) the H influx is 5 mmole/l cells hr and when pH_o = 5.80 it is 22 mmole/l cells hr. When SO_4 is substituted for Cl on both sides of the membrane the proton influx amounts to 0.2 mmole/l cells hr at pH_o = 6.5 and 2.8 mmole/l cells hr at pH_o = 5.80. The influx of Cl as measured by an unidirectional influx of ^{36}Cl from a 150 mM CholineCl, HEPES medium (+ 0.1 mM DIDS) is 34 mmole/l cells hr when pH_o = pH_i = 7.40. As the outside proton concentration increases the influx of ^{36}Cl increases simultaneously and at pH_o = 5.80 it is 60 mmole/l cells hr. The influx of ^{36}Cl stimulated by outside protons (pH_o 7.40 to pH_o 5.80) is thus 26 mmole/l cells hr i.e. as large as the proton influx. The influx of $^{35}SO_4$ from a 100 mM $MgSO_4$ (+ sucrose,HEPES,DIDS) buffer amounts to 0.1 mmole/l cells hr and is unaffected by an increase in the outside H concentration (pH_o = 5.80).The influxes of H and Cl in low pH media were not significantly affected by 0.15 mM bumetanide or 1 mM furosemide. Thus, when a proton gradient is established across the red cell membrane (pH_o=5.80, pH_i = 7.30) the magnitude of the H influx depends upon the anion present in the solution and decreases following the sequence Cl>Br>I>SO_4 and is an order of magnitude slower when SO_4 is substituted for Cl on both sides of the membrane. (This work was supported by NIH grants HL-09906 and AM-17433.)

M-PM-Pos74 AN ANION CONDUCTANCE PATHWAY IS INVOLVED IN REGULATORY VOLUME DECREASE IN HUMAN LYMPHOCYTES. B. Sarkadi, S. Grinstein, Esther Mack and A. Rothstein, The Hospital for Sick Children, Research Institute, Toronto, Ontario, M5G 1X8, Canada.

Regulatory volume decrease (RVD), evoked by the swelling of human lymphocytes in hypotonic media, is based on a rapid efflux of KCl from these cells. The selective increase in K transport is thought to be Ca-activated, inasmuch as it is blocked by Ca-depletion and quinine. Addition of the cation ionophore gramicidin to lymphocytes in isotonic media does not alter their volumes. In contrast, the ionophore completely restores RVD in Ca-depleted or quinine-treated cells swollen in hypotonic choline-Cl or Tris-Cl media. These findings indicate a volume-dependent increase in the anion conductance of the membrane. This pathway is partly blocked by DIDS and NIP-taurine and entirely abolished by dipyridamole, all powerful inhibitors of anion fluxes in red cells. RVD is also blocked by low concentrations of oligomycin either in the presence or absence of gramicidin. The volume-dependent increase in anion conductance is transient: addition of gramicidin at increasing intervals following swelling results in progressively lower rates of shrinking. The half-time for disappearance of the response is 5-8 min. (This work has been supported by grants from the Medical Research Council and the National Cancer Institute, Canada).

M-PM-Pos75 MEMBRANE NOISE AND IMPEDANCE MEASUREMENTS IN CHARA CORALLINA. Stephen M. Ross, Dept. of Botany, University of Toronto, Toronto, Ontario, Canada.

Measurements of electrical noise and frequency-dependent membrane impedance have been performed in the giant-celled alga Chara corallina. Changes of these parameters in response to pH, potassium concentration and the inhibitors fusicoccin, DCCD and NH_4^+ are described and interpreted in terms of membrane transport processes known to occur in these cells.

Of particular interest is a low frequency noise component which appears to depend on cytoplasmic streaming, and which may be removed by application of cytocholasin B. Others have shown that maintenence of the spatially separated "acid" and "base" transport regions in this cell depend on cytoplasmic streaming (see Lucas and Dainty, 1977, J. Memb. Biol. **32**: 75) and it is likely that this low frequency noise results from control of this localization.

The author wishes to acknowledge the generous support for this work provided by Dr. Jack Dainty.

M-PM-Pos76 MEASUREMENT OF ACETAMIDE FLUX IN ERYTHROCYTES WITH 360 MHz ^1H NMR.
E.G. Movius, (Intr. by A. Walter), Dept. of Med., Div. of Nephrology, U.C.S.D, La Jolla, CA 92093

Transport rates of acetamide and other nonelectrolytes have previously been determined for RBC's using osmotic and tracer methods (Sha'afi, et. al., J. Gen. Phys. 58:238, 1971). We have utilized ^1H NMR to measure acetamide transport across RBC's under equilibrium conditions. RBC's were washed twice in 150 mM NaCl, and then washed five times in a buffer containing D_2O, NaCl 125 mM, glucose 25 mM, Hepes 5 mM, & acetamide 100 mM; pD=7.0. NMR spectra at 360 MHz on suspensions at 50% Hct. exhibited two partially resolved peaks at 704 Hz and 710 Hz corresponding to the methyl resonances of acetamide present on the inside and outside respectively of the RBC. (The protein envelope of hemoglobin was suppressed using a 90-τ-180 pulse sequence.) When $MnCl_2$ 20-40 mM was added to the RBC suspensions of Hct. 28 & 50% at 22° C, the T_1 relaxation of the acetamide methyl peak could be resolved by nonlinear least squares into three components corresponding to the very rapid relaxation externally, the intermediate relaxation rate internally, and the slower relaxation rate of the hemoglobin envelope. From these rates an internal residence half-time $t_{1/2}$ = .31 ± .02 sec (P_d = 8.5 ± .08 x 10^{-5} cm/sec) was calculated which is 1.5-fold longer than that of Sha'afi et. al. (ibid.). This discrepancy is less than the 1.9-fold longer $t_{1/2}$ obtained for RBC H_2O flux by NMR vs. tracer methods (Pirkle et. al., Biophys. J. 25:389, 1979). From these studies we conclude that ^1H NMR is a convenient method for determining flux rates of rapidly exchanging nonelectrolytes such as acetamide across the RBC membrane. (Supported by NIH grant PHS 5T32 AM07249)

M-PM-Pos77 pCMBS BINDING TO THE RED CELL MEMBRANE AS CHARACTERIZED BY QUENCHING OF INTRINSIC TRYPTOPHAN FLUORESCENCE. Mark S. Tinklepaugh, James A. Dix, Michael F. Lukacovic, A.S. Verkman and A.K. Solomon, Department of Chemistry, SUNY, Binghamton, NY, and Biophysical Lab, Dept. of Physiology and Biophysics, Harvard Medical School, Boston, MA.

Mercurial reagents inhibit water and urea transport and accelerate a cation leak in the red cell membrane by mercaptide bond formation with protein sulfhydryls. We have found that mercurial reagents also quench tryptophan fluorescence of the red cell membrane and have used this quenching to study the binding of p-chloromercuribenzene sulfonate (pCMBS) to red cell ghost membranes. The concentration dependence of the total quenching after 10 min yields an apparent dissociation constant of pCMBS from ghost membranes of 0.3-0.5 mM. Pretreatment of ghost membranes with the sulfhydryl reagent, N-ethylmaleimide, does not affect appreciably the extent or concentration dependence of the quenching. The reducing agent, mercaptoethanol, rapidly and partially reverses the quenching. Kinetic studies reveal three distinct time courses with time constants of < 5 s, 1-10 min, and 40-150 min. Analysis of the first two time courses implies that the mechanism for pCMBS binding to ghost membranes consists in part of a rapid binding to the membrane (dissociation constant 1-3 mM), followed by a slow conformational change (forward rate constant 0.010 ± 0.003 s^{-1}, reverse rate constant 0.005 ± 0.002 s^{-1}). Similar pCMBS quenching characteristics were obtained by measuring tryptophan fluorescence of a major red cell protein, band 3, reconstituted into lipid vesicles (Lukacovic et al., Biochem. 20, 3145 (1981)), suggesting that our measurements probe the interaction of pCMBS with band 3 in ghost membranes. Supported in part by NIH HL29488, HL14820, HRC 14016, and MA Heart Assoc. 13-401-812.

M-PM-Pos78 EFFECT OF MERCAPTOETHANOL ON WATER AND ETHYLENE GLYCOL TRANSPORT IN HUMAN RED CELLS. B. Chasan, Y. Balkanski and A. K. Solomon (Intr. by P. Dorogi), Biophysical Laboratory, Dept. of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115 and Dept. of Physics Boston Univ., Boston, MA 02215.

It has been shown that thiourea inhibits urea permeability in human red cells (Wieth et al, Comp. Biochem. Physiol. Transp., p 317, North Holland Publ., 1974). We reasoned that mercaptoethanol (ME), which is a thio-analogue of ethylene glycol (EG), might have analogous effects on EG permeability and found that ME inhibited virtually the entire EG passive influx (375 mM conc. gradient) with a K_I of 25-30 mM, somewhat higher than the 10 mM K_I for thiourea inhibition of passive urea influx (375 mM conc. gradient). ME also inhibits water flux by 50% or more with a K_I of 70 mM. After removal of ME from the system by simple aqueous dilution, the effects were reversed, indicating that the ME effect is not due to a chemical reaction. SH compounds are significantly more lipid soluble than the OH analogues. We have previously suggested (Solomon et al, Ann. NY Acad. Sci., in press) that urea is transported into the cell through an aqueous pore with hydrogen bonding interfaces at the entrance and exit where the urea hydration shell is exchanged for hydrogen bonds within the pore. Thiourea is presumed to bind in the interchange region and inhibit the hydration shell exchange. We attribute the ME effect on EG permeability to similar interference by ME which has been absorbed to EG sites within the hydrogen bonding interchange region. The hydrogen bonding properties of water are similar to those of alcohols and we suggest that the effect of ME on water transport may be due to a similar interference with hydration shell exchange. Supported in part by NSF grant PCM-7822577.

M-PM-Pos79 INTERACTION OF ORGANIC MERCURIALS WITH THE ERYTHROCYTE ANION EXCHANGE PROTEIN: KINETICS, THERMODYNAMICS, AND INHIBITOR EFFECTS. Michael F. Lukacovic, A. S. Verkman and A. K. Solomon, Biophysical Lab., Dept. of Physiology and Biophysics, Harvard Med. Sch., Boston MA 02115

Addition of organic mercurials (p-chloromercuribenzene sulfonate, pCMBS; p-chloromercuribenzoic acid, pCMB; p-aminophenylmercuric acetate, pAPMA) and $HgCl_2$ to N-ethylmaleimide (NEM) treated red cell ghosts or band 3 vesicles results in a rapid decrease in membrane tryptophan fluorescence (< 5s; dissociation constant K_1) followed by a slower (1-10 min), concentration dependent fluorescence decrease (forward rate constant k_2, reverse rate constant k_{-2}) according to the mechanism: b3 + HgX <---> b3-HgX <---> b3*-HgX. Values for K_1 and k_2 were determined in ghosts from the concentration dependence of the slower exponential fluorescence time course. k_2 (1.0 ± 0.2 min^{-1}) did not depend on the identity of the mercurial, while K_1 varied significantly (pCMBS, 0.8 mM; pCMB, 0.2 mM; pAPMA, 0.3 mM; $HgCl_2$, 2.7 μM). Preliminary determinations of the temperature dependence of the pCMBS-band 3 conformational change in red cell ghost membranes (0.04 mg/ml protein) in 28.5 mM Na citrate, pH 7.4 suggest $\Delta H = 6.5$ kcal/mole and $\Delta S = -21$ eu for the forward conformational change (k_2), based on the slower exponential alone. $CaCl_2$ (5 mM), NaCl (150 mM), D-glucose (2 mM), urea (1 M) and 4,4'-dibenzamido-2,2'-disulfonic stilbene (DBDS, 1 mM) had no effect on the time course of pCMBS binding to red cell band 3; phloretin decreased pCMBS-band 3 binding rates with a $K_I \approx$ 1.3 μM. These experiments indicate a direct effect of mercurial inhibitors of water transport on the red cell anion transport system. Since NEM reacts with 5 of the 6 SH groups on band 3, the decrease in tryptophan fluorescence is attributed to mercurial binding to the SH group not blocked by NEM. Supported in part by NIH grant HL 14820 and the Mass. Heart Assoc. grant 13-401-812.

M-PM-Pos80 MG^{++} DISTRIBUTION IN HUMAN LYMPHOCYTES. W. Negendank and C. Shaller, Hematology-Oncology Section, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104.

Total Mg, determined by flaming cells incubated in Hank's medium containing 1 mM Mg at 37°, is 12.5 +/- 2.2 (SE,n=6) mmol/kg wet cell weight. At 0°, the cells retain ATP, are metabolically inhibited with a marked reduction in synthesis and utilization of ATP, and gain Na to a level greater than in the medium (165 +/- 3.4 mM vs 145 mM Na_{ex}). However, total cell Mg is unchanged for at least 48 hours (13.4 +/- 1.7 mmol/kg). If cell Na at 0° follows a Donnan equilibrium in absence of outward pumping of Na, then the Donnan ratio,r, is 1.1. Mg free in cell water should then rise to only 1.2 mM, and this may not be detectable by flaming. However, in cells incubated in medium low in Na (22 mM), cell Na at 0° reaches 110 mM, and if following a Donnan equilibrium would indicate a value of r of 5.0. Hence, free cell Mg should rise to 25 mM in cells in 1 mM Mg_{ex}; yet, Mg rises to no more than 11.4 mM in these cells. Cell Mg at 0° over a range of external Mg of 1 to 40 mM shows a saturable and a nonsaturable fraction. The former includes most of normal Mg and saturates at less than 1 mM external Mg. The nonsaturable fraction exists in a ratio to external Mg of 0.5. If the nonsaturable fraction mirrors Mg free in cell water, its concentration normally is about 0.5 mM, similar to that estimated by Rink et al (J.Cell Biol. 95:189,1982) by different methods (eg., P31NMR). Thus, in cells at 0° in which by a number of criteria outward "pumping" of ions is inhibited, [Mg] free in cell water remains less than in the medium, and Mg fails to follow a Donnan equilibrium. The results are explained by the concept that cell water exists in an ordered state (BBA Biomembr. Rev. 694:123, 1982) and in this state is responsible for the net exclusion of free Mg. (Supported by ONR and VA).

M-PM-Pos81 ELECTROCHEMICAL H^+ GRADIENT IN PLATELET α-GRANULES. S. Grinstein and W. Furuya. Hospital For Sick Children, Toronto, Ontario, Canada.

The internal pH and membrane potential of α-granules were determined. Fluorescence microscopy showed accumulation of weak bases, indicative of an acidic interior, inside numerous vesicles in intact platelets and in isolated α-granules. Fluorescent base uptake was external pH-dependent and NH_4^+-sensitive. A ΔpH of 1.2 was measured by [^{14}C] methylamine distribution in isolated α-granules suspended in pH 7.2 medium. Uptake of isotopic and fluorescent amines was reduced by H^+/cation exchange via ionophores and by addition of NH_4^+, but also by increasing the ionic strength, suggesting that ΔpH is partly due to a Donnan potential. Transmembrane voltage measurements by two methods indicated that in the absence of ATP, some α-granules are internally negative. When measured with ^{86}Rb, this potential could be entirely collapsed by raising the ionic strength. Addition of ATP.Mg in the absence of permeating anions made the intragranular space more positive, as expected from inward electrogenic H^+ pumping. The results are compatible with the coexistence of sealed and leaky subpopulations of α-granules. The internal acidity was generated in sealed granules *in vivo* by a H^+ pumping ATPase, whereas leaky granules display an acid internal pH only in low ionic strength media as a consequence of a Donnan potential. (Supported by the Medical Research Council of Canada.)

M-PM-Pos82 THE USE OF A CYANINE DYE AS AN INDICATOR OF MEMBRANE POTENTIAL IN YEAST. A. Peña, J.P. Pardo and M. Borbolla. Departamento de Microbiología, Centro de Fisiología Celular, Universidad Nacional Autónoma de México. Apartado postal 70-600, 04510 México, D.F., México.

The cyanine $DiSC_3(3)$ was used in an attempt to measure the membrane potential in intact yeast, by following both its fluorescence changes and its uptake by the cells. The fluorescence increased when the dye was captured by the cells in the presence of an effective energy source. However the dye seems to be taken up also by the mitochondria when these organelles are energized. The uptake of the cyanine by the mitochondria produces a quenching of the fluorescence. Both quenching and the mitochondrial uptake of the dye can be blocked by 10 uM FCCP. In cells in which the mitochondria were deenergized by FCCP in the absence of a substrate, the induced efflux of K^+ by decyltrimethylammonium, expected to generate an internal negative potential, gave rise to an increased fluorescence of the cyanine. In cells in which the dye had been taken up without the participation of the mitochondria, the addition of K^+ under conditions in which it is transported into the cell, driven by the membrane potential, there was both a decrease in the fluorescence of the dye and in the uptake. Besides, the decrease of the uptake of the dye produced by K^+ is additive to that produced by low concentrations of FCCP. The fluoresence changes and the uptake of the dye observed in cells in which the mitochondria were uncoupled, seem to be indicators of the electric potential of the plasma membrane. This membrane potential, however, is difficult to assess quantitatively. The mitochondria thus, are important to consider when measuring membrane potentials in intact yeast, and the cyanine dyes can be used as indicators of mitochondrial potential changes *in situ*. Supported by CONACyT grant No. PCCBNAL 790256

M-PM-Pos83 ENCAPSULATION OF HEMOGLOBIN IN PHOSPHOLIPID VESICLES: PREPARATION AND PROPERTIES OF SURROGATE RED CELLS. Martha C. Farmer, Bruce P. Gaber and Eddie L. Chang; Biomolecular Optics Section, Optical Probes Branch, Code 6510, Naval Research Laboratory, Washington, D.C. 20375, USA.

The development of a synthetic emergency blood replacement requires a physiologically compatible O_2 and metabolite carrier. We have encapsulated hemoglobin in phospholipid vesicles together with other buffer components in order to adjust the O_2 and CO_2 binding properties of the hemoglobin. The phospholipid mix is dimyristoyl phosphatidylcholine:cholesterol: dicetyl phosphate, in the ratio 5:4:1. The vesicles are produced by extrusion of a suspension of lipids in a buffered hemoglobin solution through a graded series of controlled pore-size membranes (Nuclepore). The procedure produces a highly monodisperse suspension of vesicles, as determined by photon correlation spectroscopy and scanning electron microscopy. The median diameter may be preselected over the range of 0.2 to 1.0 micron. Our standard preparation uses stroma-free hemoglobin prepared by us from outdated human blood (~10mM heme) in 0.05 M phosphate buffer, 140mM NaCl, 1mM EDTA, pH 7.4 (20° C). Phosphate, chloride and DPG concentrations can be readily varied to adjust the oxygen affinity as measured with a Hem-O-Scan. Our measurements show that the phospholipid vesicles are essentially unilamellar and impermeable to these anions. Stopped-flow rapid-kinetic measurements of the rate of deoxygenation of hemoglobin in vesicles indicate that this rate is correlated predictably with vesicle diameter and exceeds the rate in red cells.

M-PM-Pos85 CHARACTERIZATION OF EPR SPECTRUM OF 4-MALEIMIDE SPIN LABEL RAT CORTEX SYNAPTIC PLASMA MEMBRANES. A.E. Lunsford, M.J. McCreery, R.C. Lyon*, and C.E. Swenberg. Radiation Science Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814 (Intro by E.K. Gallin).

The spin-label method has served as a useful technique for investigating the dynamic behavior and function of biological membranes. Recent studies have shown that a nitroxide derivative of maleimide will covalently bind to erythrocyte membrane proteins to yield two components identifiable in the EPR spectrum: weakly (W) and strongly (S) immobilized. We report here an attempt to further characterize these protein components in purified (Jones and Matus, BBA 356, 296, 1974) rat brain synaptic membranes (SPM). The SPM pellet was labeled with 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl; the EPR spectrum of the labeled SPM's clearly exhibited W and S components. However, this spectrum theoretically could be fit with no fewer than 9 (3 triplets) lorentzian lines suggesting the presence of a third component. Addition of $NiCl_2$ to the suspension at 25°C showed a concentration dependent reduction of the W component to nearly zero at 0.25 M. Quickly freezing the suspension in liquid N_2 and then recording the spectrum at temperatures from -70°C to +25°C showed a strong temperature dependence of both W and S. However, a discontinuity in this dependence was observed for W with the intensity increasing sharply from near zero at the aqueous phase transition of -5° to -10°C. At -40°C, the S-component exhibited power saturation at $\sim$ 140mW. The various spin-labeled protein fractions were further characterized by the use of enzymatic and degradative agents. Digestion of SPMs with 0.125% trypsin for 30 min. decreased the protein concentration by 50% but left the W/S ratio nearly unchanged. Hydrolysis with 0.05 N NaOH (Smith and Loh, J. Neurochem., 30, 259, 1978) increased the W/S ratio while drastically decreasing the protein content. These results strongly suggest that the W component resides near the aqueous interface of the membrane while the S component is deeper within the hydrophobic region. (*NRC Research Associate)

M-PM-Pos86 ON THE SPIN LABELING OF INTACT RED BLOOD CELLS WITH COVALENT SPIN LABELS. M. Tabak *+ and N.C. Meirelles. Instituto de Fisica e Quimica de Sao Carlos, USP, 13560 Sao Carlos*; Present address Dept. Physiology, Vanderbilt University, Nashville 37232 + and Depto. Bioquimica, Instituto de Biologia, UNICAMP, S.P., Brasil.

In recent years a number of investigations have been carried on the properties of proteins of red cell membranes using the method of spin label and it is interesting that most of them have used erythrocyte ghosts. In the present investigation we have looked at the spin labeling of intact erythrocytes using the 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl spin label. With ghosts this label gives the characteristic immobilized spectrum due to its binding to protein sulfhydryls. With intact cells there is not immobilized signal and only a signal typical for a viscous solution is obtained ($\tau_c \sim 10^{-10}$sec). The maleimide spin label penetrates rapidly the cell and its correlation time is a measure of the internal cell viscosity. Using fresh cells it was noticed a reduction of the signal in the cell which is characterized by an initial rapid phase. Cells which were pretreated with N-ethyl maleimide (NEM) prior to the spin label showed an immobilized signal characteristic of labeled proteins. It was reported that NEM penetrates the red cell and is to react for instance with hemoglobin. We believe that part of the effect of NEM treatment is able to block an intracellular system so that the spin label is now available to react with the proteins both in the membrane and in the intracellular medium. (Work supported by CNP_q. We are indebted to Dr. J.H. Park and Dr. A. Beth for interesting discussions.)

M-PM-Pos87 EPR DETECTION OF ELECTROSTATIC ASYMMETRY IN LIPOSOMES AND PHOTORECEPTOR MEMBRANES. Steven A. Sundberg and Wayne L. Hubbell, Department of Chemistry, University of California, Berkeley, CA 94720

In earlier work, Castle and Hubbell (1976) demonstrated the use of a spin-labeled amphiphile as a probe for the electrostatic potential at the outer surface of charged phospholipid vesicles. In recent experiments, we have shown that the hydrophobic anion tetraphenylboron (TPB) promotes transbilayer migration of the probe molecule. Relaxation data recorded following the rapid mixing of the probe with TPB-containing vesicle samples provides information about the electrostatic potentials at both the outer and inner vesicle surfaces. The measured potentials for both surfaces of asymmetrically screened vesicles were found to be in good agreement with theoretical values calculated using their known surface charge density. The method is also sensitive to transmembrane potentials as indicated by the response of the label to potentials created with the use of potassium concentration gradients and valinomycin.

We are currently applying this technique to the study of photoreceptor membranes of the rod outer segment (ROS). Results from preliminary experiments with isolated bovine ROS, done in collaboration with Dr. Paul Schnetkamp, suggest that a large, inside-negative, electrostatic asymmetry exists in the stacked disc structures. Sonication or lysis of the outer segments destroys the observed asymmetry.

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

Castle, J.D. and Hubbell, W.L. (1976) Biochemistry 15:4818.

M-PM-Pos88 TIME RELATED IN VIVO AFFECTS OF ACETYLSALICYLIC ACID ON HUMAN ERYTHROCYTE MEMBRANES AS DETECTED WITH ELECTRON SPIN RESONANCE SPECTROSCOPY. D.L. Mazorow, A. Haug, and E.J. McGroarty. Mich. State Univ., E. Lansing, MI.

Erythrocytes from non-smoking human subjects were drawn by venapuncture prior to and 1/2 hr., 1 hr., 2 hrs., 5 hrs. and 8 hrs following ingestion of 10 grains of acetylsalicylic acid. Erythrocytes of female subjects displayed a time-dependent disordering of the membrane over the eight hour period. The level of membrane alterations may depend on steroid hormone levels. Erythrocytes drawn from females at the beginning of the estrous cycle showed the greatest amount of aspirin induced membrane disordering at one hour but by eight hours after aspirin ingestion, the membrane structure had returned to that of control. The time-dependent changes which occurred in membrane structure from subjects in the middle of the estrous cycle displayed a biphasic disordering of the membrane. An analogous study of male subjects showed a slight aspirin induced ordering of the erythrocyte membrane throughout the time span examined. Washed erythrocytes, when mixed with salicyclic acid in a bicarbonate buffer, were identical to the control cells whereas salicyclic acid added to washed erythrocytes in filtered human serum induced an ordering of the cell membrane. This study indicates that aspirin induced alterations in membrane structure may be metabolically controlled.

M-PM-Pos89 VESICULAR STOMATITIS VIRUS INDUCED FLUIDITY CHANGES IN SPIN LABELED CELL MEMBRANES: INFLUENCE OF INTERFERON (IFN). Peter L. Gutierrez, Lydia Hernaez Davis* and Raveendran Pottathil*. Developmental Therapeutics, University of Maryland Cancer Center and Department of Pediatrics University of Maryland Medical School, Baltimore, Maryland.

Human laryngeal carcinoma cells (Hep 2) were spin labeled with 3-doxylstearic acid, cooled on ice, treated with virus and kept on ice for one hour to achieve synchronization of virus penetration. The multiplicity of infection (MOI) ranged from 1-5 plaque forming units per cell(pfu/cell). Under these conditions, the membranes of the infected cells became more rigid than controls as judged by the order parameter S. Thus for VSV infected cells $S = 0.73 \pm 0.01$ (3 exp) versus $S = 0.69 \pm 0.01$ (6 exp) for control cells (Average $\pm$ SD). The order parameters were measured at room temperature 20 minutes after the sixty minute cold incubation. ^{125}I labeled VSV showed that by 20 minutes, most of the viruses have penetrated the Hep 2 cell as judged by trypsin resistent radioactive counts. These data show that virus penetration induces specific changes in membranes that renders them more rigid than controls. The increase in membrane rigidity correlates well with virus uptake by the cell. When Hep 2 cells are pretreated with human alpha interferon, the order parameter is 0.71 ± 0.01 (4 experiments). A student t test shows that this value and $S = 0.73$ for untreated infected cells are significantly different at a confidence level of $P<0.05$. Specific increases in cell membrane rigidity may be a prerequisite for the proper processing of the virion. IFN interferes with this virus induced cell membrane rigidity.

M-PM-Pos90 RAT BRAIN SYNAPTOSOMAL MEMBRANE CHANGES INDUCED BY PURIFIED MOJAVE NEUROTOXIN: AN ESR STUDY. J. Harris, T.J. Power, A.L. Bieber & A. Watts, Biochemistry Department, Oxford University, Oxford & Chemistry Department, Arizona State University, Tempe, Arizona 85287.

ESR spectra of 8 positional nitroxide labelled stearic acids & a rigid androstanol spin label, respectively, intercalated into isolated purified rat brain synaptosomal membranes were obtained as a function of temperature from 4°C to 40°C. Flexibility gradients from spin label order parameters & polarity profile from isotropic splitting factors across the synaptosomal membrane were characteristic for lipid bilayers. Labelled androstanol revealed the abrupt onset of rapid cooperative rotation about the long axis of the molecule at 12°C, showing rapid rotation of the lipid molecules along their long axis at physiological temperatures. Purified neurotoxin affected the synaptosomal membrane in a complex manner. At temperatures lower than 20°C, the neurotoxin changed the flexibility gradient of the membrane, indicative of an increased disordering in the upper regions of the membrane & increased order in the deeper regions of the membrane. The neurotoxin had little effect on the flexibility gradient of the synaptosomal membrane at 20°C, a temperature at which the acyl chain labels detected a structural change in the membranes. At temperatures greater than 20°C, the neurotoxin increased rigidity of the outer layers & fluidity of the inner layers of the membrane. A cross-over point for the neurotoxin perturbation occurred about 12-14 Å into the membrane. At all temperatures the rotational lipid motion was inhibited by the toxin as indicated by the steroid probe. ESR results are interpreted as a penetration of the neurotoxin in a manner similar to that proposed for pancreatic phospholipase A_2, a likely homologous protein. Such penetration may result in a greater ordering of the lipid chains at the outer regions but a further disordering in the inner regions of the synaptosomal membrane at physiological temperatures. Support by Wellcome Research Travel Grant (JH), Burroughs-Wellcome Foundation (AW) & NIH (ALB) are acknowledged.

M-PM-Pos91 SPIN LABEL AND DSC STUDIES OF CHL V79 CELL MITOCHONDRIAL MEMBRANES. Hisham Al-Qysi and James R. Lepock, Guelph-Waterloo Program for Graduate Work in Physics, Waterloo Campus, Waterloo, Ontario, Canada N2L 3G1.

The spin label 2,2-dimethyl-5-dodecyl-5-methyloxazolidine-N-oxide (2N14) has been used in a number of membrane studies. It has a motionally narrowed three line spectrum, allowing the calculation of a correlation time of rotation (τ) by standard methods. We show that 2N14 is weakly ordered ($S = 0.1 - 0.2$) and undergoes somewhat anisotropic motion ($\tau_C/\tau_B \simeq 1.1$) in membranes. In isotropic fluids the motion appears to be isotropic ($\tau_C/\tau_B = 1$). Arrhenius plots of τ_B and τ_C for 2N14 in mitochondria isolated from Chinese hamster lung V79 cells have inflection points, or critical temperatures, at approximately 7-10°C and 28-32°C. Similar, although fainter, inflection points are seen in plots of order parameter (S) vs. temperature for 5-doxyl-stearate and 12-doxyl-stearate in mitochondria. Thus the membrane physical changes occurring at these temperatures affect both the rate of motion and order of at least three spin labels in a consistent manner. Arrhenius plots of τ for 2N14 in whole cell homogenates and in a crude microsomal preparation also show breaks at 7-10°C and 28-32°C, implying that internal membranes other than mitochondria alone may be involved. Whole cell homogenates consistently give shorter correlation times, probably due to the presence of lipid droplets of high fluidity. DSC scans of mitochondria using a Perkin-Elmer DSC-2 show a faint, broad reversible endotherm between approximately 10 and 30°C, corroborating the spin label results and indicating that the spin label critical temperatures are due to a broad transition, probably lipid, between about 10 and 30°C.

M-PM-Pos92 A SPIN LABEL STUDY OF THE CRYSTALLINE DOMAIN OF THE URINARY BLADDER LUMINAL MEMBRANE. J. A. Vergara and D. B. Chesnut. Department of Anatomy and Department of Chemistry, Duke University Medical Center, Durham, North Carolina 27710.

Spin label experiments have been carried out on the bovine urinary bladder luminal membrane employing 5-, 7-, 12- and 16- doxyl substituted stearic acid methyl ester and compared for reference to similarly labeled bovine erythrocytes. The bladder membranes are significantly more fluid and less ordered than the bovine red blood cell membranes, but similar to liposomes composed of the lipids extracted from the erythrocytes or bladder membranes. This result suggests that the highly organized proteins of the bladder membrane act as a coat on the lipid bilayer and, while intrinsic in nature, do not perturb the lipid bilayer to an extent observable by our labeling experiments. Supported by NIH grant GM/AM28224.

M-PM-Pos93 MEMBRANE-WATER PARTITIONING OF SPIN LABELS: NO LARGE EFFECT OF PROTEINS, Betty J. Gaffney and Gary L. Willingham, Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218

The interactions of two types of spin label amphiphile with lipid membranes and with red blood cell ghosts have been examined. From paramagnetic resonance spectra, the amphiphiles studied can be divided into two categories. Fatty acid spin labels have the usual orientation: intercalated roughly normal to the membrane plane. In contrast, amphiphiles similar to the fatty acids, but with a carboxyl group at both ends of an aliphatic chain, lie on the surface of the membrane. The latter conclusion results from (1) EPR studies of oriented membranes, (2) the effect (or lack thereof) of chain length on partition coefficient and (3) the absence of a change in the partition coefficient at a synthetic lecithin phase transition.

Both classes of spin label amphiphile partition between membrane and water. A comparison of the partition coefficients (lipid/water) in dispersions of red cell lipids and in red cell ghosts gave values of 9 and 5, respectively, for a dicarboxylic acid and 495 and 678, respectively, for a monocarboxylic acid spin label. Thus, no large effect of protein on partition coefficient was found in contrast to a report by M. J. Conrad and S. J. Singer for a study of other amphiphiles in red cell lipids and ghosts (Biochem. 20, 808-818 (1981)).

M-PM-Pos94 IN SITU SPIN LABELLING OF CELL SURFACE GLYCOPROTEINS. Rob Snoek, Departments of Chemistry and Pathology, University of B.C., Vancouver, Canada, V6T 1W5.

A nitroxide spin label has been attached to the glycocalyx of native red blood cells via periodate oxidation followed by reductive amination with $NaBH_3CN$ and tempamine, a procedure reasonably specific for sialic acid residues. Under our conditions 20 ($\pm$ 10)% of the sialic acids are oxidized (CH_2O) assay. Increased oxidation causes red cells to swell and lyse. Elimination of free spin label was only possible by lysing the cells. No noticeable Heisenberg exchange ($\pm$ 2%) occurred under the conditions used. The yield was $4.0\pm1.7 \times 10^6$ spins/cells representing 65% of the oxidized sialic acids. The background signal (unoxidized red cells plus spin label) varied from 0 to 30% (15$\pm$5% avg.) of that of the modified cells, most of the background label being lipid extractable. NaOH extraction of spin labelled membranes left 91% of the spin label in the pellet which contains the membrane glycoproteins and glycolipids. The NaOH extractable spin label had a decreased t_c compared to spin labelled ghosts or the NaOH pellet and unlike the other two was insensitive to wheat germ agglutinin (WGA). Triton-X 100 extracted 76% of the spin label along with the glycoproteins and glycolipids. This extract could be separated into a WGA-precipitable component and a WGA-insensitive component with a decreased t_c compared to spin labelled ghosts. Analysis of tritiated spin labelled ghosts (using NaB^3H_3CN) on SDS-PAGE showed 50-60% of the ^3H in the PAS-1 peak and only 3-6% in the lipid peak. The covalently linked spin label on the ghosts increased its t_c with increasing WGA binding to the ghosts. Inhibition of WGA binding with GlcNAc revealed a WGA-sensitive and a WGA-insensitive spin label population. Stronger periodate oxidation produced a WGA insensitive signal.

M-PM-Pos95 THE PRESENCE OF AMILORIDE BINDING SITES IN HOMOGENIZED TROUT GILL AND KIDNEY. C. J. Costa, L. B. Kirschner, E. J. Cragoe. Dept. of Zoology, Washington State University, Pullman, WA 99164-4220, and Merck Sharp and Dohme Research Laboratories, West Point, PA 19486.

A nitroxide derivative of amiloride (cf. Briggman, J. V., Cragoe, E. J., Couch, R., Spicer, S. S., Fed. Proc. 41(5); 1694; 1982) was used to identify putative apical membranes in centrifugal fractions of gill and kidney homogenates from the rainbow trout. The tissues were homogenized in sucrose (250 mM) solution containing MOPS buffer (20 mM, pH 7.5), EDTA (5 mM) and dithioerythritol (1 mM), and the homogenates centrifuged at 10K x g(10K), 27K x g(27K) and 100K x g(100K). The pellets were resuspended in sucrose (250 mM) solution containing MOPS buffer (20 mM, pH 7.5). When the nitroxide-labelled compound (ASp, 3×10^{-5}M) was added to fractions from gill the electron paramagnetic resonance (EPR) signal was markedly reduced by the 10K centrifugal fraction, less so by 27K and 100K. Addition of amiloride (10^{-3}M) reversed the depression by the 10K fraction with little effect on the signal in 27K and 100K. This indicates that most of the membranes with an amiloride binding site (i.e. apical cell-membranes) are in the 10K fraction, which also contains most of the mitochondrial membranes. Essentially the same pattern was observed with kidney fractions. Fractions from muscle homogenates depressed the EPR signal, but amiloride had no effect in any of them.

M-PM-Pos96 IONIC BRIDGING WITHIN LIPOPOLYSACCHARIDE AGGREGATES. A pH TITRATION STUDY. Richard T. Coughlin*, A. Haug+ and Estelle J. McGroarty+. *Baylor College of Medicine, Houston, TX, and +Michigan State University, East Lansing, MI, 48824.

The head group packing of lipopolysaccharide (LPS) from Escherichia coli has been examined under conditions of varying hydroxide ion concentrations. Using the electron spin resonance probe 4-dodecyl dimethyl ammonium-1-oxyl-2,2,6,6-tetramethyl piperidine bromide as a reporter of the head group mobility, we have analyzed the structure of defined salts of LPS both from a k12 strain (strain D21) and from a deep rough mutant strain D21f2 which is missing most of the core polysaccharide. At neutral pH, the head group mobility of the magnesium salt of LPS from strain D21 was substantially less than that of the sodium salt. However, at high pH (pH 12) the head group regions of the two salts were approximately equally mobile. The changes at high pH were completely reversible precluding the possibility of alkaline hydrolysis of the LPS. Titration of the sodium salt of LPS from strain D21 with hydroxide ions indicated the existence of multiple ionizable groups above pH 7, whereas the LPS from the heptoseless mutant strain D21f2 appeared to contain a single titratable group. It is proposed that fixed charges in the core region of the LPS contribute significantly to the surface potential of the LPS aggregates and that the polysaccharide core is a region of high polarity where the pK_a of ionizable groups are substantially altered. The results reported here suggest that ionic bridging between charged groups covalently bound to the polysaccharide core region stabilize the structure of pure LPS aggregates.

M-PM-Pos97 MEROCYANINE-540 AS A SPECTRAL PROBE OF SICKLE CELL MEMBRANE CHARACTERISTICS. Neal Hermanowicz, Victor Glushko, Caterina Manna and Sangduk Kin. Department of Biochemistry and Fels Research Institute, Temple University School of Medicine, Philadelphia, PA.

Plasma membranes of sickle cell erthrocytes differ in several physical and biochemical characteristics from normal membranes. Sickle cell erythrocytes exhibit reduced methylation of membrane proteins. Merocyanine-540 (MC-540) was used to ascertain if this difference was associated with an alteration in membrane structure. Blood was obtained from normal donors and patients with sickle cell disease. Unsealed erythrocyte ghosts were prepared by hypotonic lysis and repeated washings to remove hemoglobin. Incubation of MC-540 with the ghosts produced a marked increase in extrinsic fluorescence. At the conditions used in this study, the dye is essentially non-fluorescent when free in solution. The excitation and emission maxima of 566±1 nm and 578±1 nm, respectively, reflect a relatively nonpolar environment for the dye bound to the membranes. Excitation and emission spectra of MC-540 were identical in distribution for both normal and sickle cell ghosts. However, MC-540 fluorescence from sickle cell ghosts was only 70 to 85% as intense as from normal ghosts under equivalent conditions. The relative amounts of MC-540 associated with the two types of ghosts were determined. Sickle cell ghosts were found to have only 50 to 70% of the MC-540 bound to normal ghosts. Consequently it appears highly unlikely that the effect is due to a higher quantum yield of the dye in normal ghosts. Indeed, the effective quantum yield may actually be greater in the sickle cell ghosts. Since the association of MC-540 with membranes may be influenced by electrostatic interactions, the amount of MC-540 bound to the two types of ghosts may reflect differences in distribution of surface charges on the membranes.

M-PM-Pos98 PHYSICAL ARCHITECTURE OF LIPOPOLYSACCHARIDE OF YERSINIA PESTIS. Arnold A. Peterson*, Richard T. Coughlin+, Willard T. Charnetzky# and Estelle J. McGroarty*, *Mich. State Univ., East Lansing, MI, +Baylor College of Med., Houston, TX, and #Washington State Univ., Pullman, WA.

Isolated lipopolysaccharide (LPS) of Yersinia pestis grown under different conditions was analyzed in an attempt to correlate virulence with changes in LPS structure. Two strains of Y. pestis, a calcium dependent (cal^+) and a calcium independent (cal^-) were grown either at 26° or 37°C, with or without added calcium. The levels of cations bound to LPS were measured by inductively coupled plasma emission spectroscopy and found not to vary with either growth temperature or presence of added calcium. However, native LPS from the cal^+ strain had more calcium bound than native LPS from the cal^- strain. Uniform sodium salts of the LPS isolates (Na-LPS) were made by electrodialysis followed by dialysis of the samples against Na-EDTA, pH 7.0. Electron spin resonance probing of Na-LPS with 5-doxylstearate showed no differences in acyl chain mobility among the different preparations. In contrast, the probe 4-dodecyldimethyl ammonium-1-oxyl-2,2,6,6-tetramethyl piperidine indicated that the head group region of Na-LPS from 37° grown cells is more mobile than that of Na-LPS from 26° grown cells. These results suggest a dependence of the molecular structure of the LPS head group with growth temperature but little detectable change of the acyl chains. Na-LPS from the cal^+ strain grown at 37° without calcium (limited growth) had an intermediate head group mobility, indicating an incomplete adaptation to the higher temperature. Electron microscopic examination of samples negative stained with Na-phosphotungstate revealed that the Na-LPS from cells grown at 26° exists predominantly as pitted sheets, whereas the Na-LPS of 37° grown cells exists mainly as tubes.

M-PM-Pos99 MOLECULAR STRUCTURE OF THE MAMMALIAN URINARY BLADDER MEMBRANE. K.A. Taylor, J. Vergara, and J.D. Robertson. Duke University Medical Center, Durham, N.C.

We have obtained a model of the 3-dimensional structure of the hexagonal plaques isolated from the luminal membrane of the bovine urinary bladder to a resolution of about 30 Å by combining, computationally, electron micrographs of well preserved hexagonal patches tilted from 0-60°. Our electron microscope specimens were negatively stained with neutralized phosphotungstic acid and were examined in a Philips EM 400 electron microscope equipped with a high tilt goniometer stage and low dose kit to minimize radiation damage. The structure factor data were combined satisfactorily in space group P6. The main regions of density in the map appeared to be due solely to the hexagonal protein layer on the luminal face. The structure consists of a layer about 53 Å thick which contains 12 ellipsoidal stain excluding densities: six of these densities form an inner ring surrounding a deep pool of stain on the 6-fold axis while the other six form an outer ring. When the model is viewed from that which we tentatively believe is the luminal side, the densities on the outer ring appear to be tilted at an angle of about 20° relative to the 6-fold rotation axis in a sense that is anticlockwise and slightly outwards. The densities of the inner ring are tilted to a lesser degree (12°) but in an opposite sense (clockwise). The putative molecular weight for the asymmetric unit is 47.7kd at the contour level we have chosen. At this resolution there is no evidence of penetration of negative strain through the lipid bilayer nor is any periodic structure revealed on the cytoplasmic face. Supported by GM/AM 28224.

M-PM-Pos100 EFFECTS OF LIPOSOME SIZE ON THE DEGRADATION OF LIPOSOMES IN THE MOUSE LIVER. Paul L. Beaumier and Karl J. Hwang, Department of Pharmaceutics, University of Washington, BG-20, Seattle, Washington 98195.

The relative rates of degradation of the outer lipid bilayer of large multilamellar and small unilamellar bovine brain sphingomyelin/cholesterol (2:1;mol/mol) liposomes in the liver of BALB/c mice were compared. The rate of the release of entrapped In-111 ions from the aqueous reservoir of small unilamellar liposomes (187 $\pm$42 A in diameter) or from the outermost aqueous compartment of multilamellar liposomes was used to monitor the rate of degradation of the exterior lipid bilayer surface of these liposomes. The technique of gamma-ray perturbed angular correlation (PAC) and a method for loading In-111 ions into the outermost aqueous compartment of liposomes were used in this investigation. It was found that in the liver the exterior lipid bilayer of large multilamellar liposomes was degraded more rapidly than the bilayer of small unilamellar liposomes in vivo. The average half-life for the in vivo degradation of the small unilamellar liposomes and the exterior lipid bilayer surface of the large multilamellar liposomes was estimated to be 3.55 $\pm$0.25 hr and 1.38 $\pm$0.22 hr, respectively. In contrast to the situation for small unilamellar liposomes, the degradative process for large multilamellar liposomes in the liver was not maintained under ischemic conditions. Our results suggest that multiple pathways operate in the degradation of liposomes in the liver. The rate of of degradation of liposomes in the liver may depend on accessibility of liposomes to various cellular degradative sites. (Supported by the U.S. Army Medical Research and Development Command Contract DAMD 17-78-C-8049, PHS grants AM 25608 & CA 09081 and an NSF grant PCM 81-05141).

M-PM-Pos101 ANALYSIS OF HUMAN ERYTHROCYTE GHOSTS FOR ACETYLCHOLINESTERASE, 5'-NUCLEOTIDASE AND SUPEROXIDE DISMUTASE. R.A. Zepp Johnston* and A. Petkau, Medical Biophysics Branch, Atomic Energy of Canada Research Company, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, Canada, ROE 1LO

Fresh human erythrocyte ghosts were prepared by serial hypotonic hemolysis (J. Th. Hoogeveen et al, 1970, J. Memb. Biol. 3:156-172). Residual ghost hemoglobin was 0.10 $\pm$ 0.02% of the mean corpuscular hemoglobin. The amount of membrane protein extracted was 0.66 $\pm$ 0.08 pg/ghost. The high selection factor of 1500:1 for inner membrane surface proteins over hemoglobin in the ghosts suggests a high degree of purity from cytoplasmic components. The activity of the membrane enzyme marker, acetylcholinesterase, was 1.3 $\pm$ 0.2 fmol of acetylthiocholine iodide hydrolyzed per minute per ghost, in agreement with previous results (T.L. Rosenberry et al, 1981, J. Biochem. Biophys. Methods 4:39-48). In contrast, 5'-nucleotidase specific for adenosine monophosphate was not found, again in agreement with earlier results (J. Delaunay et al, 1978, Biomedicine, 29: 173-175). Polyacrylamide gel electrophoresis of the ghost proteins, extracted with Nonidet P-40, demonstrated the presence of active cupro-zinc superoxide dismutase, in agreement with earlier results on bovine erythrocyte ghost membranes (Petkau et al, 1981, BBA 645: 71-80).

M-PM-Pos102 DYNAMIC STUDY OF FLUORESCENT PROBE MOTION AND LIFETIME IN CHL V79 CELL PLASMA AND MITOCHONDRIAL MEMBRANES. Kwan-Hon Cheng and James R. Lepock, Guelph-Waterloo Program for Graduate Work in Physics, Waterloo Campus, Waterloo, Ontario, Canada N2L 3G1.

Measurements of lifetime, steady state polarization, and differential phase polarization of diphenylhexatriene (DPH) and trimethylammonium DPH (TMA-DPH) in plasma and mitochondrial membranes from Chinese hamster lung V79 cells were made as a function of temperature using an SLM phase fluorimeter. Lifetime heterogeneity was observed for both probes in both membrane systems. A best two lifetime fit gave values of $\tau \approx 4$ and 10 nsec for DPH and $\tau \approx 2$ and 6 nsec for TMA-DPH. These values compare favorably to those made with a PRA pulse fluorimeter at room temperature. An order parameter (S) and correlation time of rotation (ρ) was calculated for both probes using an average lifetime. S was greater for TMA-DPH than for DPH in both membranes and S was greater for both probes in plasma membranes than in mitochondrial membranes. Both probes had a slightly greater value of ρ in plasma membranes. As a function of temperature, all motional parameters could be fit by a straight line in plasma membranes for both probes from 0-45°C, while discontinuities were observed at ca. 8 and 32°C in mitochondria. Thus, plasma membranes have no detectable transitions, although a broad transition from 8-32°C seems to be present in mitochondria. This mitochondrial transition correlates well with the previously observed absence of cell growth below ca. 30°C and increase in hypothermic killing below ca. 10°C in V79 cells.

M-PM-Pos103 RESTORATION OF SYNAPTIC MEMBRANE ORDER FOLLOWING CHRONIC ETHANOL TREATMENT IN MICE. By Robbe C. Lyon and Dora B. Goldstein (Intr. by Jane H. Chin), Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305.

In a previous investigation we reported changes in the order of neuronal membranes associated with the development of ethanol tolerance and physical dependence in mice (Lyon and Goldstein, 1983, Mol. Pharmacol. 23: in press). This current study monitors the fluid state of these membranes during the recovery period following withdrawal from chronic ethanol treatment. Swiss Webster mice were made physically dependent on ethanol by 3 days of continuous exposure to ethanol vapor and daily injections of pyrazole to stabilize the blood alcohol levels. Synaptic membranes (washed free of ethanol) were prepared from the brains of ethanol treated mice (E-membranes) and control mice (C-membranes) sacrificed at 0, 9, and 30 hours after ethanol withdrawal. Nine hours marks the peak of withdrawal convulsions and by 30 hours mice have completely recovered from the hyperexcitable state. The order parameter of membranes spin-labeled with 12-doxylstearic acid was measured by a sensitive EPR technique. At the time of withdrawal the E-membranes were significantly more ordered than the C-membranes. This rigid state in the absence of ethanol may be an expression of membrane dependence. By 9 hours after withdrawal, the E-membranes had partially recovered but the order was still higher than in the C-membranes. The order of E- and C-membranes prepared at 30 hours was not significantly different. The restoration of membrane order and the behavioral recovery from ethanol dependence appeared to follow similar time courses. (Supported in part by USPHS National Services Award AA05145 and Grant AA01066).

M-PM-Pos104 X-RAY DIFFRACTION OF A PURIFIED CANINE CARDIAC SARCOLEMMAL MEMBRANE. R. McDaniel, R.A. Colvin, T.F. Ashavaid, L. Herbette, Univ. of Conn. Health Center, Cardiology Division, Farmington, CT 06032

Sarcolemmal membranes with a high lipid content (6200 nmoles fatty acyl chains/mg total protein) were isolated as unilamellar vesicles from canine ventricle in the absence of calcium (< 5 μM). From assays of Na/K ATPase in the absence and presence of alamethicin or valinomycin plus potassium, 80% of the vesicles are sealed with 90% of the sealed population being right-side out. SDS gel electrophoresis shows numerous proteins ranging from 30,000 to 200,000 molecular weight. This complex protein profile is consistent with freeze fracture electron micrographs showing numerous particles of various sizes on both the concave and convex fracture faces. Lamellar meridional x-ray diffraction from hydrated oriented sarcolemmal membrane multilayers was recorded on film in which four orders indexed on an average multilayer unit cell repeat of 63 $\pm$ 1 Å. Lipids were extracted from sarcolemmal membranes and hydrated multilayers likewise yielded four diffraction orders indexing on a repeat of 62 $\pm$ 1 Å. These unit cell repeats must contain a single membrane since thin section electron micrographs show a minimal membrane width of 40-50 Å in vesicle dispersions and 30 A for fixed oriented multilayers. The lamellar intensities for both intact sarcolemmal membranes and extracted lipids were appropriately corrected and the most probable electron density profile structures compared. Both profile structures had similar features to a lipid bilayer structure with an average separation of 44 Å for the two major electron dense maxima in the single membrane profile. These maxima are presumably associated with phospholipid headgroups. We are attempting to place these profile structures on an absolute electron density scale to determine the contribution of protein to the sarcolemmal membrane structure.

M-PM-Pos105 THE EGF RECEPTOR/PROTEIN KINASE: A NEW MODEL FOR TRANSMEMBRANE SIGNALING OF HORMONE RECEPTOR OCCUPANCY. James V. Staros, Susan A. Buhrow, and Stanley Cohen. Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232.

The binding of epidermal growth factor (EGF) to its plasma membrane receptor evokes a number of cellular responses which ultimately culminate in mitosis. One of the rapid responses to the binding of EGF is the stimulation of a tyrosyl-specific protein kinase which catalyzes the phosphorylation of a number of membrane and cytoplasmic proteins. Among the membrane protein substrates of this enzyme, the most predominantly phosphorylated is the EGF receptor. We have affinity labeled the EGF-stimulable protein kinase with 5'-p-fluorosulfonylbenzoyl adenosine and have demonstrated that the kinase site resides in the same polypeptide chain as does the receptor. Therefore, the observed phosphorylation of the EGF receptor is apparently autophosphorylation. Our discovery that both the receptor and the kinase are parts of the same protein makes activation of the EGF-stimulable protein kinase the most likely candidate for the initial transmembrane signaling event on binding of the hormone. Thus, we propose the following model: The EGF-receptor/protein kinase is a transmembrane glycoprotein with the receptor site accessible from the extracytoplasmic environment and the kinase active site accessible from the cytoplasm. The two sites are in separate folding domains of the molecule, as the two activities have distinct thermal labilities; however, the two sites are allosterically coupled, since binding of EGF to the receptor site stimulates the kinase. The activation of the kinase which results from binding of the hormone to the receptor site is the primary transmembrane signaling event indicating receptor occupancy. (Supported by AM25489 and HD00700 from the NIH.)

M-PM-Pos107 MEASUREMENTS OF THE SIZE OF GLUCOCORTICOID RECEPTORS USING RADIATION INACTIVATION. Donald J. Gruol, Ellis S. Kempner and Suzanne Bourgeois. The Salk Institute, La Jolla, CA, and the National Institutes of Health, Bethesda, MD.

We have used radiation inactivation to determine the size of the glucocorticoid receptor in two lymphoma cell lines. We first compared the nuclear and cytoplasmic forms from a line (W7) exhibiting a cytolytic response to hormone. Samples were exposed to high energy electrons from a linear accelerator and the capacity to retain prebound hormone measured. The size of the cytoplasmic and nuclear forms were 79,000 and 75,000 daltons, respectively. The small difference is within the uncertainty of the measurements (+/- 10%) and suggests that there is no change in size as the receptor is activated into its DNA binding form. We have also measured the size of the nuclear form of receptor in a hormone insensitive "nuclear transfer increased" (nt^i) variant (S49 143R). This line translocates more receptor into the nucleus than wild type (68% vs. 54%) but is resistant to the cytolytic effect of the hormone. In contrast to reports indicating that the nt^i receptor was truncated (39,000 daltons), we found no significant difference between the w.t. and nt^i receptors (75,000 vs. 72,000 daltons). We confirmed the radiation results by comparing the size of the nt^i and w.t. receptors released from nuclei by nuclease. Based on sedimentation rates, both receptors have the same size. In addition, both were found binding to mono and multi nucleosome containing chromatin fragments. Despite its increased DNA affinity, the nt^i receptor was, however, released from nuclei at a lower ionic strength than wild type. This suggests that the nt^i may be deficient in its ability to specifically interact with nuclei, possibly through a non-DNA component. (Supported by grants from the Whitehall Foundation and the NIH.)

M-PM-Pos108 SPECTRAL PROPERTIES OF A MEMBRANE BOUND FLUORESCEIN PROBE. Susan G. Stanton and John C. Owicki, Dept. of Biophysics & Medical Physics, U.C. Berkeley, Berkeley, CA 94720.

Probe molecules tethered to phospholipid head groups have been used in several laboratories to study molecular interactions at membrane surfaces. In some cases the probe associates with the membrane more closely than one would expect. We report here the spectroscopy of a new fluorescent membrane probe, fluorescein-chlorotriazine-triglycine-DPPE. The spectral properties of this probe are a sensitive function of environment, providing direct information on probe location. The DPPE-fluorescein spacer is approximately 1.6 nm, and the probe is strongly bound by anti-fluorescein antibodies (Petrossian, Kantor, and Owicki, this meeting). The probe spectral properties are a function of temperature, probe concentration (except at low lateral densities), and membrane lipid composition. The monoanion-dianion pK_a is 1 to 2 units higher than the free fluorescein value, depending on lipid composition. The pK_a is shifted by the addition of charged lipid in qualitative agreement with Gouy-Chapman theory. The probe dianion excitation maximum in 2:1 DMPC:cholesterol membranes is red-shifted ~2 nm from the fluorescein value with only one probe environment detected. In DMPC bilayers the dianion excitation maximum is red-shifted approximately 6 nm, and the spectrum consists of at least two components. The red-shifted component of the probe excitation spectrum is similar to that of hexadecanoylaminofluorescein in DMPC bilayers, and its intesity increases upon the addition of palmityltrimethylammonium ion; thus the probe population with the red-shifted absorption is hypothesized to be closely associated with the choline groups in the lipid head group region.

M-PM-Pos109 INTERACTIONS BETWEEN MONOCLONAL ANTIBODIES AND FLUORESCENT HAPTENS THAT ARE BOUND TO THE SURFACES OF LIPID BILAYERS. Ashot Petrossian, Aaron B. Kantor, & John C. Owicki, Dept. of Biophysics & Medical Physics, U.C. Berkeley, Berkeley, CA 94720.

Ligand-receptor interactions at cell surfaces are one of the primary processes in biological recognition. We have devised a model system for studying an immunologically important example of this phenomenon, the binding of antibodies to antigens on cell membranes. The hapten, fluorescein, was covalently coupled to the head group of DPPE via a triglycine spacer, giving the fluorescein-chlorotriazine-triglycine-DPPE structure shown at the right. Small amounts of this were incorporated into phospholipid bilayer vesicles of varying composition (including DMPC, DPPC, and cholesterol). Monoclonal anti-fluorescein IgG was isolated from hybridomas generously given by E. Voss (D. Kranz et al. 1981. J. Biol. Chem. 257:6987-6995). Monovalent Fab fragments were produced by papain digestion. Antibody-hapten interactions were analyzed continuously in the physiologically important nanomolar concentration range by monitoring the hapten fluorescence, which is quenched by antibody binding. The kinetic and equilibrium properties of the binding of both IgG and Fab depend on the composition and "fluidity" of the membranes. Including cholesterol in the membranes increases both the rate and strength of the binding. In all cases studied, the initial binding rate of IgG exceeded that of Fab. The binding equilibrium was analyzed for DPPC:cholesterol (2:1) membranes; the avidity of IgG binding was greater than that of Fab. The observed differences between IgG and Fab binding probably reflect bivalent vs. monovalent binding to the membranes.

M-PM-Pos110 PYRINE-FLUORESCAMINE: A NEW MEMBRANE-BOUND DYE PAIR. C.S. Owen, Dept. of Biochemistry, Jefferson Medical College, Philadelphia, PA 19107.

Pyrene and Fluorescamine are conveniently available dyes whose spectral properties are well suited for the observation of resonant energy transfer quenching of pyrene fluorescence and enhancement of fluorescamine emission. Both dyes were bound to hydrophobic anchors which held them in a liposomal membrane to create a system whose fluorescence would be sensitive to dilution of the lipid (e.g. by fusion of the labelled liposomes with other unlabelled membranes). Commercial pyrenesulfonylphosphatidylethanolamine (PSPE) was used for the energy donor and a conjugate (FPE), of fluorescamine and phosphatidylethanolamine was used as the acceptor. The latter was synthesized by a simple one-reaction protocol. The PSPE donor was incorporated into liposomes at low concentrations to avoid excimer fluorescence. When FPE acceptor molecules were also included, quenching of PSPE fluorescence was observed which depended on the amount of FPE employed. An enhancement of FPE emission, relative to similar liposomes lacking PSPE, was also observed. Both effects depended on the close physical proximity of donor and acceptor molecules in the membrane: when vesicles were fused with unlabelled membranes or when nonionic detergent was added to increase the average PSPE-FPE separation, these spectral effects could be reversed. The sharp emission peaks from PSPE and their separation from the FPE emission band makes quantitation of quenching and enhancement straightforward. Another advantage of fluorescamine as an acceptor is that it can be reacted with integral membrane proteins as well. Energy transfer has been observed between PSPE and labelled cell surface proteins in vesicles that were generated by the detergent dialysis technique. (This research was supported in part by NIH grant RO1 AI17143).

M-PM-Pos111 SPECIFIC BINDING OF THIOCYANATE TO GASTRIC MICROSOMES. Tushar K. Ray and Jyotirmoy Nandi, Department of Surgery, SUNY-Upstate Medical Center, Syracuse, NY 13210.

Previous report (Arch. Biochem. Biophys. 216:259-271, 1982) from this laboratory on the mechanism of SCN^- inhibition of vesicular H^+ transport suggested a competition between K^+ and SCN^- for some low affinity (S_{50}%19 mM) K^+ binding site responsible for the gastric H^+, K^+-ATPase mediated vectorial transport of H^+. However, no specific binding of SCN^- to gastric microsomes has thus far been demonstrated and this is the basis of this report. Binding of $[^{14}C]SCN^-$ to gastric microsomes at pH 7.0 was studied using the millipore filtration technique. Maximum binding was observed within 30 sec at 21°C and showed a linear relationship with increasing microsomes. Ethanol wash of the filters did not reduce the membrane associated radioactivity to any significant extent suggesting that some membrane protein and not the lipid phase was primarily involved in the observed SCN^- binding process. ATP and/or Mg^{+2} did not have any effect on SCN^- binding. The binding of SCN^- was hyperbolic with a K_a of 10 mM. Scatchard analysis of the data was linear suggesting a single class of binding sites and the maximum was about 5 n moles/mg protein. The steady-state level of SCN^- binding was reduced to about 50 and 90% in presence of 50 and 100 mM K^+ respectively; while Na^+ at concentrations up to 150 mM was without any effect. Furthermore, the anions like Cl^-, SO_4^{-2}, NO_3^- and gluconate were unable to affect SCN^- binding. The data are compatible with our hypothesis that SCN^- inhibits gastric H^+ transport by interfering with a low affinity K^+ site within the domain of the gastric H^+, K^+-ATPase system.

M-PM-Pos112 INTERACTION BETWEEN MONOVALENT ANIONS AND A DISULFONIC STILBENE IN BINDING TO THE RED CELL MEMBRANE. Kevin R. Smith and James A. Dix, Department of Chemistry, SUNY, Binghamton, NY.

The inhibitor of red cell anion exchange, 4,4'-dibenzamido-2,2'-disulfonic stilbene (DBDS), binds to the anion transport protein of the red cell membrane, band 3, via a bimolecular step (dissociation constant 3 μM), followed by a slow conformational change of band 3 (forward rate constant 4 /s, reverse rate constant 0.09 /s); the binding is accompanied by a fluorescence enhancement of DBDS (Dix et al., Nature 282, 520 (1979)). We have studied the effect of a series of monovalent anions (HCO_3^-, F^-, Cl^-, Br^-, and I^-) on DBDS binding to unsealed red cell ghost membranes at constant ionic strength. All the anions apparently compete with DBDS for binding to band 3, as measured by equilibrium DBDS fluorescence. The dissociation constants for the anions obtained from these measurements are (in mM) I^-, 1; HCO_3^-, 1; Br^-, 5; Cl^-, 5; F^-, 5. Fluorescence stopped-flow experiments resolve the total binding into a bimolecular step and a conformational step. The monovalent anions affect both the bimolecular step and the conformational step of DBDS binding. The dissociation constants for the bimolecular step in the presence of 10 mM monovalent anion are (in μM) 3 (Cl^-), 5 (HCO_3^-), 7 (Br^-), 10 (I^-), and 16 (F^-). The forward rate constants for the conformational change are (in s^{-1}) 10 (Cl^-), 12 (HCO_3^-), 16 (Br^-), 23 (I^-), and 38 (F^-). These results imply that there is a nonspecific anion binding site on band 3, that anions and DBDS can be simultaneously bound to band 3, and that inhibition of anion transport by DBDS may occur via a conformational change in band 3. Supported by NIH HL29488.

M-PM-Pos113 CHARACTERIZING THE CATECHOLAMINE CARRIER OF CHROMAFFIN GRANULE MEMBRANE WITH CATECHOLAMINE RECEPTOR LIGANDS. R.B.Kropf and E.W.Westhead, Department of Biochemistry, Univ. of Massachusetts, Amherst, MA 01003.

Norepinephrine (NE) and epinephrine (E) uptake into, and release from intact chromaffin granules (CG) or resealed ghosts (RG), can be simultaneously monitored using HPLC with electrochemical detection. We have found that ATP:Mg enhanced NE uptake into intact CG of bovine adrenal medulla in the presence of added 200 μM NE is not coupled with release of E. This observation suggests that NE uptake is not an ATP:Mg activated exchange with internal E, as proposed by Ramu et al. (J.B.C. 256, 1229-1234, 1981). We have examined the kinetics of NE uptake into RG, measured an apparent K_m of approximately 10 μM, and determined the effect of several catecholamine receptor ligands on NE uptake. Irradiation of CG membrane in the presence of 50 nM ^{3}HNE resulted in the labeling of several membrane proteins, particularly a doublet of molecular weight 28000 - 32000, as revealed by SDS PAGE and fluorography. Phenoxybenzamine (PBZ) is an α adrenergic antagonist which binds irreversibly. We have examined the effect of PBZ on NE uptake into RG. In the presence of 10 μM NE, with no preincubation of vesicles (0.2 mg protein/ml) with PBZ, an inhibition with IC_{50} of approximately 2 μM was observed. With a 3 min preincubation, apparent V_{max} of NE uptake decreased by 60%, while the apparent K_m was unchanged. Increase of inhibition with time of preincubation with PBZ, as well as the effect on V_{max} and K_m, suggest that an irreversible interaction of PBZ with the catecholamine transporter may be taking place. The binding of ^{3}HPBZ to lysed CG membranes is currently being explored to confirm the labeling pattern of membrane proteins observed with ^{3}HNE. [Supported by NIH research grants GM 14945 and GM 24197.]

M-PM-Pos114 STOPPED-FLOW STUDIES OF MYELIN BASIC PROTEIN (MBP) ASSOCIATION WITH PHOSPHOLIPID VESICLES. P.D. Lampe, G.J. Wei, and G.L. Nelsestuen. Department of Biochemistry, University of Minnesota, St. Paul, Minnesota, 55108.

When mixed with vesicles containing acidic phospholipids, MBP causes vesicle aggregation. The kinetics of this vesicle cross-linking by MBP was investigated using stopped-flow light scattering. The process was highly cooperative requiring about 20 protein molecules per vesicle to produce a measurable aggregation rate and about 35 protein molecules per vesicle to produce the maximum rate. The maximum aggregation rate constant approached the theoretical vesicle-vesicle collisional rate constant. Vesicle aggregation was second order in vesicle concentration and was much slower than protein-vesicle interaction. High MBP concentrations did not inhibit vesicle aggregation indicating that vesicle cross-linking occurred through protein-protein interactions. In contrast, poly-L-lysine-induced vesicle aggregation was inhibited by high peptide concentrations indicating that it did cross-link vesicles as a peptide monomer. The MBP:vesicle stoichiometry required for aggregation and the low negative free energy for protein dimerization suggested that multiple cross-links were needed to form a stable aggregate. Stopped-flow fluorescence was used to estimate the kinetics of MBP-vesicle binding. This reaction was much faster than vesicle aggregation, and the half-times obtained suggested a rate constant which approached the theoretical protein-vesicle collisional rate constant. (Supported in part by NIH HL 15728)

M-PM-Pos115 CHARACTERIZATION OF MATRIX-BOUND BAND 3, THE ANION EXCHANGE PROTEIN FROM HUMAN ERYTHROCYTE MEMBRANES. Reinhart A.F. Reithmeier and Amechand Boodhoo, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Band 3 (M_r = 95,000), the anion transport protein of human erythrocyte membranes, is a dimer in solutions of non-ionic detergents such as octa-ethylene glycol mono n-dodecyl ether ($C_{12}E_8$). The role of the dimeric structure of Band 3 in the binding of 4-benzamido-4'-aminostilbene-2,2'-disulfonate (BADS), an inhibitor of anion transport (K_i = 2 μM), was studied by characterizing the interaction of BADS with dimers and monomers of Band 3 covalently attached to p-chloromercuribenzoate-Sepharose 4B. Matrix-bound Band 3 dimers bounds BADS with an affinity of 1 to 3 μM at a stoichiometry of 0.7 to 1.0 moles of BADS per mole of Band 3 monomer, in agreement with the BADS binding characteristics of Band 3 in the membrane. This binding site could not be detected if Band 3 had been isolated from cells pretreated with 4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS), an irreversible inhibitor of anion transport. By limiting the amount of p-chloromercuribenzoate on the Sepharose bead, Band 3 dimers could be attached to the matrix by one subunit only. Matrix-bound Band 3 monomers were formed by dissociation of these dimers by 0.25% dodecyl sulfate at 0°. Refolded matrix-bound Band 3 monomers, formed by complete removal of the dodecyl sulfate, were unable to bind BADS. Band 3, dissociated to the monomer in solution, was unable to bind BADS after attachment to the matrix and removal of the detergent. Removal of the dodecyl sulfate from Band 3 in solution allowed reformation of the dimer with recovery of the ability of Band 3 to bind BADS. These results suggest that the BADS binding sites (two per dimer) are at the interface between two halves of the dimer. (Supported by Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada).

M-PM-Pos116 CYTOCHROME b_5 INDUCED FLIP-FLOP OF PHOSPHOLIPIDS IN SONICATED PHOSPHOLIPID VESICLES: EVIDENCE FOR TRANSIENT BILAYER DESTABILIZATION UPON INITIAL BINDING. Susan F. Greenhut* and Mark A. Roseman, Uniformed Services University of the Health Sciences, Dept. of Biochemistry, 4301 Jones Bridge Road, Bethesda, MD 20814.

We have been studying the effect of cytochrome b_5, an integral membrane protein, on the flip-flop of phospholipids in sonicated vesicles. Flip-flop was monitored by chemical titration of external amino lipid (Roseman, et al., Biochemistry 14,4826 (1975)). Vesicles prepared from 9:1 phosphatidylcholine:phosphatidylethanolamine were amidinated with isethionylacetimidate, creating an asymmetric distribution of PE across the bilayer. 3-10 mol amidinated cyt b_5 were incubated per mol amidinated vesicles for 40 min in 0.01 $NaHCO_3$ 10^{-4} EDTA, pH 7.4 at 25° or 37°, and flip-flop of PE then monitored by reaction with trinitrobenzenesulfonic acid. Typical results (below) show that initial interaction with protein causes a transient burst of lipid flip-flop, followed by a very slow rate of flip-flop: Outside/total PE before amidination, 0.583; after amidination, 0.117; 40 min after addition 10 cyt b_5, 0.211; 18 hr after addition of 10 cyt b_5, 0.250. No flip-flop takes place without cyt b_5. There are two explanations for this transient bilayer destabilization. (1) The water soluble conformation of cyt b_5 has detergent-like properties, (2) initial interaction causes a mass imbalance across the bilayer that is relieved by lipid flip-flop. Subsequent experiments support the mass-imbalance explanation. Transient bilayer destabilization may be required for post-translational insertion of many membrane proteins, especially those which span the membrane. (NIH # AM 30432-01 and USUHS # C07117).

M-PM-Pos117 DO "SALT-BRIDGES" EXIST IN MEMBRANE PROTEINS? Barry Honig and Wayne Hubbell, Department of Biochemistry, Columbia University, New York, N.Y. 10032 and Department of Chemistry, University of California, Berkeley, CA 94720.

Sequence analysis of a number of transmembrane proteins suggest that "charged" amino acids may be located in the interior of the phospholipid bilayer. It is of interest to consider whether these residues will form ion-pairs ("salt-bridges") as they do in the interior of water soluble proteins, or whether they will tend to appear in neutral form. To this end, we discuss the energetics of proton transfer reactions in low dielectric environments. Our approach is to use gas phase proton affinities together with the Born approximation to arrive at an expression for the free energy of proton transfer between a carboxyl and an amino group. We find that a closely associated ion-pair will be more stable than the neutral species suggesting that buried salt bridges will be formed in membrane proteins if an appropriate pair of residues are located close to one another in space. We summarize a number of experimental observations which indicate that ionic species are in fact stable in low dielectric media. Moreover, the stability of ion-pairs is likely to be much greater in membrane proteins than in a homogeneous dielectric due to the numerous possibilities of hydrogen bonding to adjacent polar groups in proteins. Comparing the relative stability of ionic and neutral residues embedded in a low dielectric has relevance with regard to structure and function of membrane proteins. The question of the energy required to remove these residues from water bears on the mechanism of insertion of proteins into membranes. We discuss means of estimating the free energy of hydrogen bonded ion pairs in membranes relative to that of the solvated ionic species in water. The energetics of inserting charged residues into membranes are discussed in light.

M-PM-Pos118 LATERAL DIFFUSION OF THE VESICULAR STOMATITIS VIRUS GLYCOPROTEIN IS SIGNIFICANTLY SLOWER IN THE NUCLEAR MEMBRANE OF INFECTED CELLS THAN IT IS IN THE PLASMA MEMBRANE. L. Puddington, D.S. Lyles, K. Jacobson, and J.W. Parce, Bowman Gray School of Medicine of Wake Forest University and University of North Carolina, Chapel Hill.

Fluorescence recovery after photobleaching (FRAP) was used to measure lateral diffusion of the vesicular stomatitis virus encoded glycoprotein, G, in the nuclear membrane of infected Friend erythroleukemia cells. Fluorescent Fab fragments of monoclonal antibodies directed against G, and dihexadecyl indocarbocyanine (dil) which measures diffusion of lipids, were used as probes in FRAP experiments. The diffusion coefficient (D) of G in the plasma membrane of infected cells was $\sim 5 \times 10^{-10}$ cm^2/sec, in agreement with values reported by others in different cell types (1,2). Diffusion of G in the nuclear membrane of nuclei isolated from infected cells was not detected, ($D < 5 \times 10^{-12}$ cm^2/sec). However, diffusion of nuclear membrane lipids was detected, $D \sim 3 \times 10^{-9}$ cm^2/sec. These results present a striking difference in viral glycoprotein diffusion between nuclear and plasma membranes. The extremely slow diffusion of the G protein in the nuclear membrane suggests an extensive and quantitative interaction of this protein with some other cellular constituents, possibly cytoskeletal in nature.

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M-PM-Pos119 THE EFFECT OF pH ON THE INTERACTIONS IN MIXED MONOLAYERS BETWEEN PHOSPHATIDYLETHANOLAMINES AND ALL-TRANS RETINAL. P. Tancrède, S. Robert, C. Désilets and R.M. Leblanc, Université du Québec, Centre de recherche en photobiophysique, Trois-Rivières (Québec), Canada G9A 5H7.

The interactions in mixed monolayers between distearoyl-L-phosphatidylethanolamine (PE(18:0)), natural phosphatidylethanolamine (PE) purified from bovine rod outer segments and all-trans retinal have been studied at the nitrogen-water interface at 21.0 ± 0.5°C. Seven mixtures of each phospholipid with all-trans retinal, covering the whole range of molar fractions, were studied. The monolayers were spread on a phosphate buffer subphase 10^{-3} M at three different pH values, 5.5, 7.1 and 8.2. The surface phase rule shows that all-trans retinal is miscible with the natural phospholipid at the interface. Small, negative deviations with respect to the additivity rule are observed and small, negative excess free energies of mixing are calculated for this system. On the other hand, when PE(18:0) is mixed with all-trans retinal, the components are no longer miscible at the interface. Also, the isotherms for both sets of mixtures are not shifted when the subphase pH is varied from 5.5 to 8.2, which suggests that no Schiff base is formed between retinal and PE at the interface, under the present experimental conditions. This results from both the apolar nature of all-trans retinal, and the conformation of the polar ethanolamine group at the interface. When extrapolated to the *in vivo* system, this implies that within the disk bilayer, the retinal isomers would also interact hydrophobically within the aliphatic portion of the membrane, away from the polar heads of the phospholipid molecules. Our results therefore raise questions about the existence of a Schiff base between PE and the retinals *in vivo*.

M-PM-Pos120 THE EFFECTS OF THE PHYSICAL STATES OF PHOSPHOLIPIDS ON THE PROTEIN INCORPORATION AND CYTOCHALASIN-B BINDING ACTIVITY OF HUMAN ERYTHROCYTE MEMBRANE PROTEINS IN RECONSTITUTED VESICLES. Jong-Sik Hah, S.W. Hui and Chan Y. Jung, Biophysics Department, Roswell Park Memorial Institute, Buffalo, N.Y. 14263 and Biophysics Laboratory, Veterans Administration Medical Center and Department of Biophysical Science, State University of New York, Buffalo, N.Y. 14215.

Proteoliposomes were reconstituted from a Triton extract of human erythrocyte membrane proteins and a mixture of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of varying ratio. With mixtures of various ratios of egg PC and soybean PE, the protein/lipid ratio of the reconstituted vesicles was at a maximum at 25% egg PC and 75% PE, the composition which is known to have a maximum bilayer instability (highest occurrence of lipidic particles seen by freeze fracture electron microscopy). With the mixtures of 1-palmitoyl-2-oleoyl PC and dilinoleoyl PE, which has few isolated lipidic particles at room temperature, the effect was less pronounced. The specific activity of cytochalasin B (CB) binding protein in the reconstituted vesicles was monotonically increased up to several fold as PC content was increased in the egg PC/soybean PE mixture. Similar increase was also observed when soybean PE was partially substituted by dimyristoyl PC, cholesterol, or transphosphatidylated PE from egg PC. These findings indicate that, while preexistence of defects in lipid bilayer promotes the protein-incorporation into the bilayer during reconstitution, it is the bilayer stability that activates the CB binding protein once they are incorporated in the vesicle.

M-PM-Pos121 RAMAN SPECTROSCOPIC STUDY OF THE EFFECTS OF COLISTIN-PHOSPHOLIPID INTERACTIONS ON BILAYER ORGANIZATION. Ernest Mushayakarara and Ira W. Levin, Laboratory of Chemical Physics, NIADDK, National Institutes of Health, Bethesda, Md. 20205; and Yokio Kimura, Mukogawa Women's University, Nishinomiya, Japan.

In order to assess the effects on membrane organization derived from intrinsic and extrinsic polypeptide:lipid associations, we examined the Raman spectra of various liposomal preparations reconstituted from intact colistin (polymyxin E), a cyclic polycationic polypeptide antibiotic, and several of its fragments. The bilayer systems included 1,2-dimyristoylphosphatidylcholine (DMPC), 1-myristoyllyso PC, 1,3-dipalmitoyl PC, dimyristoylphosphatidic acid (DMPA), 1-palmitoyl-2 oleoyl PC and 1-O-hexadecyl-2-acetoyl-sn-glycero-3-phosphorylcholine. Fragments of colistin included (I) deacyl colistin, (II) colistin octapeptide, (III) colistin nonapeptide, and (IV) 6-methyl-octanoyl-L-diaminobutyric acid-threonine. Temperature profiles, constructed from C-C and C-H stretching mode parameters, reflected changes in the lipid hydrocarbon chain order/disorder characteristics. For example, for DMPA at high pH, assemblies containing either intact colistin or its separate fragments in 10:1 lipid:polypeptide mole ratios displayed temperature profiles that indicated a decrease in T_m by ∿15°C for DMPA:colistin bilayers, (b) considerable broadening (∿20°C) of the phase transition with a decrease in T_m by ∿10°C for DMPA:deacyl colistin, (c) ∿10°C broadening of the phase transition with a decrease in T_m by 4°C for DMPA:colistin octapeptide, and (d) an increase of ∿4°C in T_m for DMPA:fragment IV. Various Raman spectral parameters allowed distinctions to be made concerning the order/disorder characteristics along specific portions of the lipid acyl chains.

M-PM-Pos122 CARBON-13 NMR T_1 AND T_{1rho} STUDIES OF PROTEIN-LIPID INTERACTIONS IN SARCOPLASMIC RETICULUM MEMBRANES. A.J. Deese*, E.A. Dratz*, L. Hymel and S. Fleischer, *Division of Natural Sciences, University of California, Santa Cruz, CA, 95064 and Department of Molecular Biology, Vanderbilt University, Nashville, TN, 37235.

^{13}C-NMR T_1 and T_{1rho} relaxation times were measured for the well resolved lipid resonances of highly purified and functional sarcoplasmic reticulum (SR) membranes and pure SR lipids. Unsonicated samples were used, although sonication was observed to have little or no effect on the observed T_1 and T_{1rho} relaxation times. Single exponential T_1 and T_{1rho} relaxation rates were observed for all of the lipid resonances in the absence and presence of protein. A 15 to 25% reduction of the lipid T_1 times was observed in the presence of protein. The largest decrease observed was for the vinyl resonance. T_{1rho} times were also reduced in the presence of protein, but by a larger amount (30-45%) with greatest effect again seen for the vinyl resonance. T_1 and T_{1rho} times were observed to increase with increasing temperature. These results suggest that the calcium pump protein of SR is reducing both high frequency and low frequency lipid motions. The largest effect being the reduction of low frequency motions associated with unsaturated lipid resonances. (Work supported in part by NIH grants EY05607 to A.D., AM14632 and AM21987 to S.F. and EY00175 to E.D.)

M-PM-Pos123 DYNAMIC ORGANIZATION OF MEMBRANES CONTAINING TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR. J.F. Ellena, M.A. Blazing, P.A. Jorgensen and M.G. McNamee, Dept. of Biochem. and Biophys., University of California, Davis, CA 95616.

Reconstituted membranes containing purified Torpedo acetylcholine receptor (ACHR) and dioleoylphosphatidylcholine (DOPC) were prepared by the cholate dialysis technique to give lipid to protein ratios of 100 to 400 (mol/mol). Spin labeled lipids were incorporated into the membranes and ESR spectra were recorded between 0 to 20°C. The spin labels used included derivatives of: stearic acid (16-FASL), androstane (ASL), phosphatidylcholine (PCSL), phosphatidylethanolamine (PESL), and phosphatidylserine (PSSL). The phospholipid spin labels contained 16-doxystearic acid in the sn-2 position. All the spectra contained a relatively "immobile" and a relatively "mobile" component. Spectra of each spin label in vesicles containing only DOPC had only a mobile component. The relative amounts of the immobile and mobile components were quantitated by spectral subtraction and integration techniques. Plots of the mobile/immobile ratios versus the lipid to protein ratio gave straight lines from which the relative binding affinity of each spin label for the ACHR and the number of "immobilized" lipids per ACHR were obtained according to the procedure of Brotherus et al. (Biochemistry 20, 5261-5267 (1981)). All the spin labels gave similar values for the number of immobilized lipids (40±7). This value is close to the number of lipids that will fit around the intramembranous perimeter of the ACHR. The affinities of the spin labeled lipids for the ACHR relative to DOPC were: ASL ∿ 16-FASL (4.1) > PESL (1.1) > PCSL (1.0) > PSSL (0.7). The role of the lipid interactions in ACHR ion permeability function will be discussed. (Supported by USPHS Grant 13050.)

M-PM-Pos124 THE EFFECT OF A PHASE TRANSITION ON PENETRATION OF PHOSPHOLIPID MONOLAYERS BY MELITTIN AND GLUCAGON. H.S.Hendrickson, P.C.Fan, D.K.Kaufman, and D.E.Kleiner. Intr. by E.A.Dennis. Department of Chemistry, St. Olaf College, Northfield, MN 55057.

The penetration of melittin and glucagon into phospholipid monolayers was studied by measuring compression isotherms of phospholipids in the absence and presence of various concentrations of protein in the subphase. Differences in molecular area were calculated as a function of protein concentration at constant pressure. Area change as a function of surface pressure at constant protein concentration was also calculated. Melittin showed greater affinity for penetration into phosphatidylglycerol (PG) than into phosphatidylcholine (PC). The cutoff pressure for melittin penetration was 45 mN/m with PC and 60 mN/m (extrapolated) with PG. Dipalmitoyl PC and PG monolayers show phase transitions upon compression at 25°C. Both melittin and glucagon showed increased penetration as measured by area change within the region of the phase transition with both lipids. Glucagon showed a cutoff pressure of 25 mN/m for penetration into dimyristoyl PC. The preference of glucagon for interaction with lipid bilayers in the gel phase will be discussed with respect to monolayer penetration as a function of surface pressure. (This work was supported by a grant, NS 11777, from the National Institutes of Health)

M-PM-Pos126 RESONANCE ENERGY TRANSFER ASSAY OF PROTEIN MEDIATED LIPID TRANSFER BETWEEN VESICLES. J. Wylie Nichols and Richard E. Pagano, Dept. of Physiology, Emory Univ. School of Medicine, Atlanta, GA and Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, MD.

Resonance energy transfer between 7-nitro-2,1,3-benzoxadiazol-4-yl (NBD) acyl chain-labeled lipids (the energy donors) and (lissamine) Rhodamine B-labeled phosphatidylethanolamine (N-Rh-PE) (the energy acceptor) was used to monitor the rate of protein mediated transfer of the NBD-labeled lipids between two populations of phospholipid vesicles. The transfer rates and lipid specificity of bovine liver phosphatidylcholine specific transfer protein (PC-TP) measured with the resonance energy transfer assay were found to be similar to those obtained using radiolabeled phospholipids. In addition, when the resonance energy transfer assay was used to determine the rates of transfer of phospholipids mediated by bovine liver nonspecific lipid transfer protein (nsL-TP), the relative rates of acceleration in the presence of the protein were found to be the same as that determined using radiolabeled phospholipids (phosphatidic acid > phosphatidylcholine > phosphatidylethanolamine). The nsL-TP dependent transfer of a series of NBD-labeled lipids was found to be highly correlated with their spontaneous rates of transfer over a range of spontaneous rates spanning three orders of magnitude. Since spontaneous intervesicular transfer of NBD-labeled lipids has been demonstrated to occur via the convection of soluble monomers through the aqueous phase (Nichols, J. W. and Pagano, R. E. (1982), Biochemistry 21:1720-1726), we propose that nsL-TP functions by increasing the rate of the spontaneous transfer process by binding to the vesicle surface and nonspecifically increasing the rate of monomer-vesicle dissociation and association.

M-PM-Pos127 AN INFRARED SPECTROSCOPIC STUDY OF THE pH DEPENDENT SECONDARY STRUCTURE OF BRAIN CLATHRIN. James S. Vincent, Clifford J. Steer and Ira W. Levin. NIADDK, NIH, Bethesda, Md. 20205

Coated vesicles (CV), present in almost all eukaryotic cells, have been implicated as a major shuttle mechanism involved in the intracellular transport of membrane proteins. The lattice material surrounding the CV's is formed predominately from a 180,000 MW subunit protein structure called clathrin. The vesicle coat is easily dissociated by mild alkaline pH (8.5) to form clathrin trimers known as triskelions. At pH 6.5, these triskelions are capable of polymerizing into cage-like structures whose lattices of hexagons and pentagons are similar in size and shape to those on CV's. In an attempt to understand the structural changes of the soluble triskelions as they assemble into cages (pH 6.5), we have examined the secondary structures of these species using the amide I and II bands in the infrared spectrum. CV's were isolated from bovine brain by D_2O/sucrose gradient centrifugation (Nandi, et al, PNAS 79:5881 (1982)). Clathrin triskelions were subsequently dissociated from the vesicle membrane by 10mM Tris, pH 8.5. A decrease in the peak height intensity ratio of the amide I to amide II band from 2.5 to 1.5 and a shift in the amide I frequency from 1648 to 1656 cm^{-1} suggest a decrease in α-helix content as the triskelions polymerize to form cages. Other spectral features of the amide bands suggest the presence of both β-sheet and β-turn structures. The amide I and II bands reflecting the clathrin portion of the intact CV are nearly identical to the amide spectra of the clathrin cages polymerized from triskelions in the absence of the vesicle membrane. The results suggest that (1) there is a marked change in the secondary structure of clathrin triskelions as they polymerize to form cages at pH 6.5, and (2) the reconstituted cages serve as an appropriate model for the examination of structural changes of the clathrin coat lattice of CV's.

M-PM-Pos128 PROSTACYCLIN INHIBITION MEDIATED BY MACROMOLECULES AT THE CELL SURFACE Ray M. Price, Georgetown University Medical Center, Washington, D.C., 20007

Prostacyclin (PGI_2), an important haemostatic regulator, is normally released by human umbilical artery segments *in vitro*. This release can be reduced by components of human serum. The inhibition could not be accounted for when serum prostaglandin binding was measured. Serum was divided into albumin and serum minus albumin (S-A) fractions by affinity chromatography. (S-A) was subdivided into fractions of $< 60,000$ MW and $> 60,000$ MW by gel filtration. At a concentration of 4.4 mg/ml all fractions inhibited PGI_2 release to the same degree. Other macromolecules (polyglutamic acid (MW 50,000-100,000), polylysine (MW 70,000-150,000), polyglutamic acid + polylysine, or dextran (MW 70,000) - all 4.4 mg/ml) also inhibited PGI_2 release to the same degree. In subcellular enzyme preparations serum fractions fail to uniformly inhibit prostaglandin production. Since macromolecular cell entry is unlikely in the time scale of these experiments, the inhibitory effects of these macromolecules are probably exerted from the outside of the cell. This is consistent with the hypothesis that the macromolecules prevent lateral diffusion of membrane proteins which could prevent the components of the phosphatidyl ethanolamine methyltransferase system from associating in the cell membrane, the first step in prostaglandin production, ultimately resulting in inhibition of PGI_2 synthesis.

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M-PM-Pos129 CIRCULAR DICHROISM AND INFRARED OF CYTOCHROME OXIDASE AND OF Ca^{+2}-ATPase ORIENTED IN MULTILAMELLAR FILMS. M.D. Bazzi and R.W. Woody, Dept. of Biochem., CSU, Ft. Collins, CO 80523.

Oriented multilamellar films of purified cytochrome oxidase (CCO) and of Ca^{+2}-ATPase have been obtained by the isopotential spin-dry method (Clark, Biophys. J. 31,65, 1980). The orientation of the secondary structure of these proteins has been examined by circular dichroism (CD) and polarized infrared (IR). In the CD spectrum of CCO films, the 208nm band observed in the solution spectrum is greatly diminished, while the 222nm and 195nm bands are reduced in intensity, with the latter shifted to 198nm. In the Ca^{+2}-ATPase film spectrum the 208nm band is almost completely eliminated and the 192nm band is reduced in magnitude. The solution and the film spectra have been deconvoluted into gaussian components. This analysis indicates that the helix axes are tilted by 35 deg. in CCO and 25 deg. in Ca^{+2}-ATPase, with respect to the normal. The CD spectra also indicates that both proteins contain some β-sheet. IR linear dichroism has been measured with the plane of the film normal or tilted by 60 deg. with respect to the incident beam. The amide I of CCO is increased while amide II shows little change in the tilted position in comparison with the normal position. In the case of Ca^{+2}-ATPase films, amide I is increased while amide II is decreased in the tilted position. These results indicate that the helices of CCO and Ca^{+2}-ATPase are predominantly oriented normal to the plane of the film. CCO films show a strong amide V band at 700 cm^{-1} and weaker amide V bands at 650 and 600 cm^{-1}. These bands are attributable to β-sheet, random and α-helical structures, respectively. The amide I of Ca-ATPase has two shoulders at 1690 cm^{-1} and 1630 cm^{-1} indicating the presence of β-structure in Ca^{+2}-ATPase. (Supported by USPHS grant, GM22994.)

M-PM-Pos130 A POSSIBLE ROLE FOR PHOSPHOLIPIDS IN THE ACTIVITY OF PSEUDOMONAS CYTOCHROME OXIDASE. Y. Ching, Bell Laboratories, Murray Hill, NJ 07974 and David C. Wharton, Department of Biology, Northeastern University, Boston, MA 02115.

Preparations of cytochrome oxidase (nitrite reductase) purified from Pseudomonas aeruginosa (B.B. Muhoberac and D.C. Wharton, J. Biol. Chem., in press) contain variable, but significant, amounts of phospholipids. These were removed with chloroform:methanol (2:1) from the insoluble residue after extraction of heme d_1 from the protein with acidified acetone (K.E. Hill and D.C. Wharton, 1978, J. Biol. Chem. 253, 489-495). Thin-layer chromatography of the chloroform:methanol extracts on silicic acid using a chloroform:methanol:water (65:25:4) system resulted in the separation of three components which were tentatively identified as cardiolipin, phosphatidylcholine, and phosphatidylethanolamine. Phospholipids could also be extracted from the native enzyme using Triton X-100 leading to a 90-95% inhibition of the oxidase activity. These results suggest a possible role for phospholipids in the catalytic activity of the enzyme. (Supported by NIH Grant HL29555 to D.W.).

M-PM-Pos131 INTERACTIONS OF MODEL PEPTIDES WITH MICELLES: EFFECT OF MEMBRANE-LIKE MICROENVIRONMENTS ON CONFORMATION, Jeffrey E. Lacy, Department of Chemistry, University of Delaware, Newark, DE 19711

The influence of membrane microenvironments on polypeptide chain folding has been investigated through the interaction of model peptides with surfactant micelles. Several peptides that are only slightly soluble in water have been solubilized in aqueous sodium dodecyl sulfate (SDS) solution to determine the effects of the ordered, hydrophobic micelle interior and the surfactant-water interfacial region on peptide conformation. Comparison of nuclear magnetic resonance (NMR) and circular dichroism (CD) spectral parameters of the peptides in micellar solutions with those in bulk solvents is used to determine conformation. In addition, NMR techniques such as paramagnetic shift reagent titrations and nuclear Overhauser enhancement (NOE) measurements locate the peptides in the micelle and complement conformational analysis. Results indicate preferential orientation of the peptides relative to the micelle surface and differential conformational effects on parts of the peptides dependent on their location within the micelle.