**T-AM-Po45** PHYSIOLOGICAL SHEAR STRESSES ENHANCE THE $Ca^{2+}$ PERMEABILITY OF HUMAN ERYTHROCYTES. D.E. Brooks, Department of Pathology, F. Larsen, S. Katz and B. Roufogalis, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada V6T 1W5

Mammalian erythrocytes in vivo are bathed in a medium containing approximately $10^{-3}$M $Ca^{2+}$ while their intracellular free $Ca^{2+}$ levels are probably less than $10^{-6}$M. The trans-membrane concentration gradient is maintained by an ATP-dependent membrane-bound $Ca^{2+}$ pump which can extrude $Ca^{2+}$ from the cells at a rate of 4 to 30 x $10^{-3}$ moles $Ca^{2+}$ (liter cells)$^{-1}$ $h^{-1}$ under optimal conditions, a rate which is very much greater than the passive influx of about 6 to 10 x $10^{-6}$ moles $Ca^{2+}$ (liter cells)$^{-1}$ $h^{-1}$. The question therefore arises as to why such a potentially powerful pumping mechanism has evolved in red cells if its only role is to remove the relatively minor amounts of $Ca^{2+}$ introduced intracellularly by leakage of the magnitude reported to date. Our results suggest that $Ca^{2+}$ permeability and hence pump activity is higher in the circulation than that measured in the laboratory in the absence of flow. We find that when red cells are exposed to physiological levels of shear stress in a rotational viscometer the passive permeability to $Ca^{2+}$ in ATP-depleted cells is increased by up to an order or magnitude concomitant with a strong enhancement in the $Ca^{2+}$-stimulated ATPase activity. In ATP-rich cells, an identical enhancement in $Ca^{2+}$ permeability is observed when 0.2mM $La^{3+}$ is included to block the $Ca^{2+}$ pump. Hence in vivo, where red cell membranes are continually deformed and stressed by fluid mechanical forces, $Ca^{2+}$ permeability may be considerably greater than is currently recognized.

**T-AM-Po46** INTERACTIONS BETWEEN EXTERNAL PROTONS AND THE ANION TRANSPORTER OF HUMAN ERYTHROCYTES. MILANICK, M.A. AND R.B. GUNN. Dept. of Biophysics and Theoretical Biology, Univ. of Chicago, IL. 60637 and Dept. of Physiology, Emory University, Atlanta, GA 30322.

Proton inhibition of chloride exchange was studied over a large range of external proton and chloride concentrations at 0°C. We measured the rate of efflux of $^{36}Cl$ from normal, intact erythrocytes ($Cl_{in}$=110 mM, $pH_{in}$=7.6) into isotonic solutions containing KCl, K-gluconate, and 27 mM glycylglycine. The external pH was varied from 4.1 to 8.0. Evidence for three different proton complexes was found: (a) $TH_0$, the proton bound to the unloaded transporter, (b) $TClH_0$, the proton bound to the transport-site-loaded transporter, and (c) $TCl_2H_0$, the external proton is bound to the transporter when both the substrate-inhibition-site and transport-site are loaded. The chloride concentration required for the peak flux decreased from 100 mM at $pH_0$=6.8 to 10 mM at $pH_0$=4.1. Thus complex $TCl_2H_0$ exists. This complex is formed at higher pH values than the other protonated complexes so that conventional inhibition analysis was impractical in attempting to determine if $TH_0$ (competitive) or $TClH_0$ (uncompetitive) or both (mixed) occurred. The ratio of Vmax-out/$K_{½}$-out (estimated from the slope of flux vs. $Cl_0$ (<3 mM)) decreased as the external proton concentration increased and thus the complex $TH_0$ exists. We were unable to ascertain directly if $TClH_0$ existed. We assumed that iodide is a substrate analog of chloride. We found that iodide and protons were not mutually exclusive inhibitors of chloride exchange and thus $TIH_0$ probably exists. This suggests that complex $TClH_0$ may also exist. We conclude that at least three transporter conformations can be protonated from the external solution. Supported in part by USPHS grants HL-20365 and GM-28893.

**T-AM-Po47** MUTUAL INTERACTIONS OF REVERSIBLE INHIBITORS ON THE RED CELL ANION TRANSPORTER.

O. Fröhlich and R.B. Gunn. Dept. Physiology, Emory University School of Medicine, Atlanta, GA 30322

Phloretin, NAP-taurine (N-(4-azido-2-nitrophenyl)-2-aminoethyl sulfonate), DNDS (2,2'-dinitro-4,4'-stilbene disulfonate) and sulfate were tested for their inhibitory effect on chloride exchange in intact human erythrocytes at 0°C and pH 7.8. Initial tracer chloride efflux was measured as function of the initial phloretin concentration in the efflux medium and at several different fixed concentrations of extracellular chloride, $Cl_0$. Phloretin was a mixed-type inhibitor with the apparent inhibitor constants $K_i$=1.2 µM and $K_i'$=2.2 µM. Chloride fluxes were also measured with combinations of each pair of the above inhibitors. The data were analysed by Dixon plots with 1/v vs. the concentration of the first inhibitor at several different fixed concentrations of the second inhibitor and constant $Cl_0$ throughout, to test whether the two inhibitors could bind at the same time (intersecting lines) or were mutually exclusive (parellel lines). It was found that sulfate, DNDS and NAP-taurine were all mutually exclusive in their binding to the anion transporter. DNDS was also mutually exclusive with phloretin, but neither sulfate nor NAP-taurine interfered with the binding of phloretin. These data can be interpreted in terms of a steric interference scheme between the different inhibitors in the anion binding pocket of the transporter. The loci of NAP-taurine, DNDS and sulfate binding mutually overlap, and the locus of phloretin only overlaps with the DNDS locus. Since DNDS and sulfate are known competitive inhibitors of chloride binding to the transport site and since phloretin is a mixed inhibitor, the phloretin binding locus is adjacent to but not overlapping with the chloride transport site. Supported by US PHS grants HL-20365 and GM-28893.

**T-AM-Po48** BUMETANIDE INHIBITION OF (Na + K + 2Cl) CO-TRANSPORT AND K/Rb EXCHANGE AT A CHLORIDE SITE IN DUCK RED CELLS: MODULATION BY EXTERNAL CATIONS. M. Haas and T.J. McManus, Department of Physiology, Duke University Medical Center, Durham, N.C. 27710

Catecholamines stimulate two distinct ion transport processes in duck red cells: (1) an electrically neutral (Na + K + 2Cl) co-transport, and (2) a K/K (or K/Rb) 1:1 forced exchange, which also requires the presence of Cl. Co-transport shows a saturable dependence on Cl with an apparent $K_{\frac{1}{2}}$ of 75 mM, whereas Rb influx via exchange has a linear dependence on Cl up to 500 mM. Both processes are inhibited by furosemide, or bumetanide. Increasing Cl concentration from 20 mM to 100 mM at a constant ratio of (internal Cl/external Cl) increases the apparent $K_i$ for bumetanide, suggesting competition between the drug and Cl for a common binding site. In contrast, raising the external concentration of Rb, $(Rb)_o$, or Na, $(Na)_o$, decreases $K_i$ for bumetanide. When co-transport was studied in the presence of a sub-maximal inhibitory dose of the diuretic (1 μM), the enhancing effect of external cations on inhibition of co-transport was half-maximal at 4.2 mM $(Rb)_o$, 14.6 mM $(Na)_o$. These values are very close to those previously reported for the apparent $K_{\frac{1}{2}}$ for stimulation of co-transport influx: 5.4 mM for $(Rb)_o$, and 17.1 mM for $(Na)_o$ (Schmidt and McManus, J. Gen. Physiol. 70: 81-97, 1977). Bumetanide inhibition of K/Rb exchange is similarly enhanced by increasing $(Rb)_o$ with both processes affected to the same extent at a given dose. These results suggest positive cooperativity between the cation sites of (Na + K + 2Cl) co-transport - and also that involved in K/Rb exchange - and the Cl site to which bumetanide binds. This Cl site also appears to have identical properties in both exchange and co-transport, despite the marked difference in the kinetics of the Cl dependence of these modes. (Supported by NIH grant GM-07171 & N.C. Heart Association)

**T-AM-Po49** CALCIUM TRANSPORT IN INSIDE-OUT DOG RED CELL MEMBRANE VESICLES. Ortiz, Olga E. and Sjodin, R. A., Department of Biophysics, University of Maryland, Baltimore, Maryland.

Vesicles from dog red cell membranes prepared by the method of Lew et al (J. Physiol. 307, 36-37 P, 1980) were 50% inside-out as judged by acetylcholinesterase accessibility. (ATP+Mg)-dependent Ca transport in these vesicles has the following features: Ca uptake is linear up to 10 min. and is stimulated by calmodulin. Uptake of Ca is abolished by $10^{-6}$ M A23187 Ca-ionophore, inhibited by $10^{-3}$ M La or $25 \times 10^{-3}$ M Mg, but is not affected by $10^{-4}$ M ouabain. The ATP-dependent Ca uptake is hyperbolically activated by external Ca with $K_{0.5}$ = 5 μM and Vm = 340 μmoles Ca/1.R.B.C./hr. Initial uptake rate is higher in both Na and K solutions than in sucrose.

Ca uptake in the absence of ATP accounts for only about 10% of the total Ca uptake, is inhibited by La or Mg, and is higher in the absence of Na.

Addition of Na causes release of the Ca accumulated by the vesicles in the presence of ATP and Ca is accumulated to lower concentrations in the presence of Na than in the absence of Na.

The results provide evidence for the presence of an ATP-dependent Ca "pump" plus a Na-Ca exchange system in the membrane of dog red cells.

**T-AM-Po50** TEMPERATURE -DEPENDENCE OF THE EFFECT OF OUABAIN ON STEADY-STATE K AND NA EXCHANGES IN HUMAN LYMPHOCYTES. W. Negendank and C. Shaller, Hematology-Oncology Section, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104. (Support by ONR and VA).

We reported (Biophys.J.33:4a,1981) that between 37 and 10° K and Na levels remained normal but steady-state K exchange decreased markedly while Na exchange remained normal. Na exchange then decreased markedly below 10°. To examine this dissociation between K exchange and Na exchange, cells were treated with ouabain, which is assumed to inhibit a membrane Na,K-ATPase pump that maintains normal Na exclusion and K accumulation. Ouabain ($10^{-6}$-$10^{-5}$M) has a maximal effect on ion levels within 24 hours, causing loss of K and gain of Na. Steadt-state $^{22}Na$ and $^{42}K$ exchanges were determined simultaneously by an efflux technique in cells pre-equilibrated 24 hours with and without ouabain. At all temperatures ouabain caused Na to rise to 150 mmol/kg and K to fall to 15 mmol/kg. At 37° the rate constant of Na exchange decreased slightly in presence of ouabain, but steady-state Na flux rose from 2.1 to 38.8 mmol/kg/hr. The temperature-dependence of Na exchange behaved in the Arrhenius plot as if there were now a single steep $E_a$ over the entire temperature range. The Arrhenius plot of ion fluxes showed a remarkable feature: in presence of ouabain Na exchange resembled normal K exchange (single $E_a$ of 12 kcal/mol) while K exchange resembled normal Na exchange (flat with indeterminate $E_a$). Clearly, the effect of ouabain on steady-state ion exchanges is not simply to inhibit a pump, or a pump along with other exchange modes of the pump, and to thereby reveal properties of remaining passive exchange mechanisms; this could not explain the increased Na flux at higher temperature nor its acquiring a more marked temperature-dependence. Rather, ouabain induces a fundamental alteration in the intrinsic properties of Na exchange in lymphocytes.

T-AM-Po51 p-CHLOROMERCURIBENZENE SULFONATE (pCMBS) INTERACTION WITH BAND 3 IN RED CELL MEMBRANES. Michael F. Lukacovic, A. S. Verkman, James A. Dix, M. Tinklepaugh and A. K. Solomon, Biophysical Lab., Harvard Med. Sch., Boston, MA and Chem. Dept., SUNY, Binghamton, N.Y. 13901

Band 3 contains 6 SH groups, 5 of which react with N-ethylmaleimide (NEM) (Rao, J. Biol. Chem. 254, 3503, 1979; Ramjeesingh et al, BBA, 599, 127, 1980). Equilibrium binding using NEM treated ghosts shows that pCMBS binds to the cryptic (non-NEM reactive) SH group on band 3 with a stoichiometry of $0.8 \pm 0.3 \times 10^6$ sites per cell with $K_d = 0.02 \pm 0.01$ mM at 0°C; gel electrophoresis using 203-Hg-pCMBS confirms that this site is on band 3. pCMBS competes with a specific inhibitor of band 3 mediated anion exchange, 4,4'-dibenzamido-2,2'-disulfonic stilbene (DBDS) with $K_I = 0.25 \pm 0.06$ mM at 23°C. Stopped-flow kinetic studies, in which DBDS is mixed with ghosts in the presence of pCMBS, show that pCMBS competitively inhibits the DBDS induced conformational change in band 3 with $K_I = 0.15 \pm 0.03$ mM at 23°C. This pCMBS effect is reversed with 5 mM cysteine or 5 mM glutathione. pCMBS quenches tryptophan fluorescence (excitation 290 nm; emission 330 nm) in ghost membranes treated with 2 mM NEM with half maximal quenching at [pCMBS] = $0.21 \pm 0.02$ mM; quenching is reversed with 5 mM mercaptoethanol. At 0.2 mM pCMBS, the decrease in tryptophan fluorescence occurs with a half time of 20 min at 23°C. pCMBS is known to inhibit over 80% of osmotic water flow at 23°C in NEM treated red cells with $K_I = 0.1$-$0.2$ mM and a half-time for development of inhibition of $\tilde{}$ 15-20 min (Chasan and Solomon, private comm.). These findings indicate that the cryptic SH group resides on band 3 and suggest that the 15-20 min half-time for inhibition of water-flow is related to a membrane protein conformational change. Supported by NIH grants 5 R01 GM15692 and 5 R01 HL14820.

T-AM-Po52 THE AQUEOUS PORE IN THE RED CELL MEMBRANE: BAND 3 AS A CHANNEL FOR ANIONS, CATIONS, NON-ELECTROLYTES AND WATER. A. K. Solomon, B. Chasan, James A. Dix, Michael F. Lukacovic, M. R. Toon and A. S. Verkman, Biophysical Laboratory, Harvard Medical School, Boston, MA 02115

It is suggested that a dimer of the anion transport protein, band 3, provides an 9 Å diameter aqueous channel through the red cell membrane - a common pathway for anions, cations, non-electrolytes and water. This conclusion is supported by several lines of evidence. The SH reagent, p-chloromercuribenzene sulfonate (pCMBS), which binds to a cryptic (not reactive with N-ethylmaleimide) SH group in the membrane, inhibits osmotic water flux by 80% and also affects binding of the specific anion transport inhibitor, DBDS, to the anion transport site on band 3 (see previous abstract). Addition of pCMBS to NEM treated red cells induces a substantial net cation leak as does the addition of other organic mercurials having known inhibitory effects on water transport; these inhibitors also inhibit DBDS binding to band 3. The effects of pCMBS on the anion system and cation and water transport all occur with a half value of [pCMBS] $\tilde{}$ 0.1-0.2 mM at 23°C. In order to show that the induced cation leak is related to band 3 rather than (Na, K)-ATPase or other membrane proteins, preliminary experiments were carried out with purified band 3 (Lukacovic et al, Biochem. 20, 3145, 1981), which contains no (Na, K)-ATPase activity and is reconstituted into PC vesicles. Addition of 0.2 mM pCMBS to these band 3 vesicles increases the cation leak by 30%, consistent with a common band 3 pathway for anions and cations. Thiourea, a non-electrolyte, reverses the pCMBS inhibition of DBDS binding to band 3 with a $K_D$ of 10 mM, $\tilde{}$ equal to the concentration at which thiourea half-inhibits urea transport. An α-helical model of the transport region of band 3 which is consistent with these observations is suggested. Supported by NIH grants HL14820, GM15692 and NSF grant PCM-7822577.

T-AM-Po53 EFFECT OF THIOUREA ON pCMBS INHIBITION OF WATER TRANSPORT IN RED CELLS. B. Chasan, M. R. Toon and A. K. Solomon, Dept. of Physics, Boston University, Boston, MA 02215 and Biophysical Laboratory, Harvard Medical School, Boston, MA 02115

The sulfhydryl reagent, pCMBS, and the nonelectrolyte, thiourea, appear to have different effects on solute and solvent flux through the human red cell membrane. pCMBS reduces both the hydraulic permeability coefficient, $L_p$ (Macey and Farmer, BBA 211, 104, 1970), and nonelectrolyte flux by 80-90 %, whereas thiourea inhibits urea (Wieth et al, Comp. Biochm. Physiol. Transp., p. 317, North-Holland Publ., 1974) and hydrophilic amide fluxes but has no effect on $L_p$. In view of the thiourea effect on pCMBS binding to band 3 (see previous abstract) we looked for a thiourea effect on pCMBS inhibition of water flux and found that thiourea inhibits the pCMBS effect on $L_p$ in a time-dependent manner. Normally a 15-20 min induction period is required for pCMBS to exercise its full effect on $L_p$. When 0.1 M thiourea is added at 0 time, 2 mM pCMBS produces no effect on $L_p$. If 0.1 M thiourea is added 5-20 min after 2 mM pCMBS, $L_p$ remains fixed at the value attained by that time. 50 % inhibition of the pCMBS effect on $L_p$ is produced by 2 mM thiourea, as compared to 10-20 mM required for 50 % inhibition of urea permeability. Normally 5 mM cysteine, added 10-20 min after pCMBS, reverses the inhibition of water flux with a reversal half time of $\tilde{}$ 5 min. Addition of 2 mM thiourea together with 5 mM cysteine increases the half time for reversal to $\tilde{}$ 12 min. These experiments show that thiourea modulates the pCMBS interaction with the site which controls osmotic water transport. There is a similar 10-20 min delay in the development of the net cation leak induced by pCMBS and 0.1 M thiourea causes this delay to increase by a further 10-20 min. These studies provide further evidence for linkage among the sites controlling: osmotic water flow, nonelectrolyte diffusion and the induced cation leak. Supported by NSF grant PCM-822577.

**T-AM-Po54** STOPPED-FLOW AND TEMPERATURE-JUMP MEASUREMENTS ON BIOLOGICAL SYSTEMS: EFFECTS OF HETEROGENEITY, UNSTIRRED LAYERS AND MULTIPLE REACTION INTERMEDIATES. James A. Dix and A. S. Verkman, Dept. of Chemistry, SUNY, Binghamton, N.Y. 13901 and Biophysical Laboratory, Harvard Medical School, Boston, MA 02115

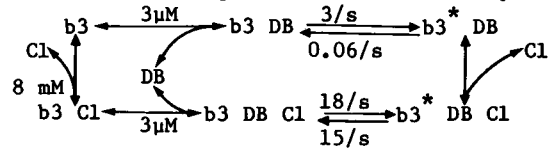
The stopped-flow and temperature-jump techniques can be used as complimentary methods to examine the mechanism of a biological reaction. In ideal systems, the two methods should yield identical reaction mechanisms and rate constant values. Under certain conditions, when the stopped-flow and temperature-jump methods give apparently contradictory results, a careful analysis of data obtained by both methods gives additional information not obtained by either method alone. The time course of any reaction measured by either method can be calculated numerically for a given mechanism and set of rate constants; a chi-squared analysis compares the computer simulated reaction time course with experimental data to select best fit rate parameters and mechanisms. Unstirred layer effects on reaction time course are calculated by solving the coupled system of kinetic and diffusional equations; heterogeneity effects are determined by approximating rate constant or size distributions as discrete values and solving the resultant coupled system. The presence of unstirred layers or sample heterogeneity can lead to large errors in the interpretation of kinetic data, especially in stopped-flow experiments at low concentrations; temperature-jump measurements are relatively insensitive to these artifacts. A comparison of stopped-flow and temperature-jump data gives an estimate of unstirred layer thickness and the degree of heterogeneity. A systematic method to explore possible additional reaction steps is given. These techniques are demonstrated for the binding of transport inhibitors to red cell membrane proteins. Supported by NIH grant 2R01 HL14820-10.

**T-AM-Po55** EFFECT OF LIPID PERTURBANTS ON RED CELL BAND 3 CONFORMATIONAL STATES. Stuart A. Forman, A. S. Verkman, James A. Dix and A. K. Solomon. Biophysical Laboratory, Harvard Medical School, Boston, MA 02115.

The concentration dependent effects of membrane anesthetics, including halothane and the n-aliphatic alcohols, on the interaction between the specific inhibitor of anion exchange, 4,4'-dibenzamido-2,2'-disulfonic stilbene (DBDS), and the membrane anion transport protein, band 3, were studied by stopped-flow fluorescence spectroscopy. The initial steps of DBDS binding to band 3 are a bimolecular association (dissociation constant, $K_1$) followed by a slow conformational change (rate constant $k_2$; Nature 282, 520, 1979). Each anesthetic increases $k_2$ in a dose dependent manner and does not alter $K_1$ in the concentration range studied (halothane, 0-25 mM; ethanol, 0-1.6 M; butanol, 0-0.2 M; hexanol, 0-25 mM; octanol, 0-3 mM). A plot of $k_2$ vs solution halothane concentration gives a straight line (r=0.99) with slope, 71 $M^{-1} s^{-1}$ and intercept, 2 $s^{-1}$. A plot of $k_2$ vs membrane alcohol concentration (partition coefficients taken from Seeman, Pharmac Rev, 24, 583, 1972) falls closely along the same straight line for all 4 alcohols (r=0.8) with slope, 9.9 ± 2 $M^{-1} s^{-1}$ and intercept, 2.0 ± 0.2 $s^{-1}$; a plot of $K_1$ vs membrane alcohol concentration has slope, -0.2 ± 0.3, not significantly different from zero. Membrane alcohol concentrations ranged from 0 to 0.5 M. These data demonstrate a specific, concentration dependent, effect of membrane anesthetics on the rate of the conformational change of the band 3-DBDS complex. The dependence of $k_2$ on membrane alcohol concentration, and not on the particular identity of the alcohol, suggests that a protein-alcohol interaction is responsible for the effect on $k_2$, rather than a non-specific membrane fluidizing effect. Supported by NIH grants GM15692 and HL14820.

**T-AM-Po56** A NON-COMPETITIVE 'SHUNT' PATHWAY FOR THE EFFECT OF CHLORIDE ON THE BAND 3-DBDS CONFORMATIONAL CHANGE IN RED CELL MEMBRANES. A. S. Verkman, James A. Dix and A. K. Solomon, Biophysical Lab., Harvard Med. Sch., Boston, MA 02115 and Chem. Dept., SUNY, Binghamton, N.Y. 13901.

The effect of Cl on 2,2'-dibenzamido-4,4'-disulfonic stilbene (DBDS) binding to band 3 in red cell ghost membranes was studied in buffer [NaCl (0-600 mM) + Na citrate] at constant ionic strength (160 or 600 mM), pH 7.4, 25°C. Equilibrium binding by fluorescence enhancement titration shows that Cl decreases the affinity of the high affinity DBDS binding site on band 3 from 0.065 μM (0 Cl) to 1.45 μM (550 mM Cl) with half saturation [Cl] = 480 mM. Stopped-flow kinetic experiments of DBDS binding to ghost membranes gave a single-exponential time course. At constant Cl, a plot of stopped-flow time constant vs 1/[DBDS] gave a straight line with $K_1$ = slope/intercept and $k_2$ = 1/intercept, assuming DBDS binds to band 3 by a rapid bimolecular association (equilibrium constant, $K_1$) followed by a conformational change (rate constant $k_2$; Nature 282, 520, 1979). $K_1$ did not depend on Cl, while $k_2$ increased from 3/s (0 Cl) to 18/s (500 mM Cl) with half-saturation [Cl] = 10-30 mM. These equilibrium and rate constants are incorporated into a shunt model (b3 = band 3; DB = DBDS); the 4 additional parameters in the shunt pathway were determined self consistently from the 5 constraints given above. These experiments demonstrate distinct binding sites for Cl and DBDS which interact in a non-competitive manner. For [Cl] < 200 mM, the shunt model predicts linear Hunter-Downs plots for DBDS inhibition of Cl self exchange, consistent with observed effects (Knauf, Curr. Top. Membr. Trans. 12, 249, 1979). Supported by NIH grant GM 15692.



**T-AM-Po57** EFFECT OF THE SURFACE CHARGE ON THE KINETIC PARAMETERS OF CATION TRANSPORT IN YEAST. Borbolla, M. Peña, A. and Theuvenet, A. Departamento de Bioquímica, Facultad de Medicina UNAM, Departamento de Microbiología Centro de Investigaciones en Fisiología celular, UNAM and Dept. of Chemical Cytology, Faculty of Science University of Nijmegen, Nijmegen The Netherlands.

Washing yeast cells with high ionic strength (300 mM $M^+$) and low pH (pH 3.0), produces an increase of surface charge with a concomitant release of bound calcium from the membrane. We found a lower Km for the uptake of $Ca^{+2}$, $Rb^+$ and ethidium bromide in cells with higher surface charge in agreement with theory. However, there is also an effect on Vmax for the uptake of $Rb^+$ due perhaps to a depolarization of the cell by the washings. We found also a much higher effect of ethidium bromide on potassium release in the cells with higher surface charge, as expected. Respiration, proton pumping and ion content of the cells are not affected by the washings. The measurements of the surface charge was carried out by using 9-amino acridine fluorescence changes.

**T-AM-Po58** EFFECT OF VOLUME CHANGES ON PASSIVE PERMEABILITY TO CATIONS IN HUMAN RED CELLS. Norma Adragna, Mitzy Canessa, and Daniel C. Tosteson; Harvard Medical School, Department of Physiology & Biophysics, Boston, MA 02115.

We have studied the effect of osmotic and isosmotic changes in cell volume on ouabain- and furosemide-insensitive (FI) cation movements in human red cells. In the osmotic procedure, cells of normal volume were incubated in hyposmotic, isosmotic and hyperosmotic medium. In the osmotic procedure, the intracellular ion content was decreased or increased by the PCMBS loading technique. Subsequently, the cells were incubated in a medium of 300 mOsM. In cells containing high K and Li replacing Na, the passive (ouabain and furosemide insensitive) permeability for K ($k_K$, $hr^{-1}$) increased from 0.014 ± 0.002 to 0.024 ± 0.001 ($p < 0.005$) when cell water content increased by isosmotic swelling from 65.8 to 68% w/w. $k_{Li}$ increased from 0.011 ± 0.001 to 0.023 ± 0.003 ($p < 0.005$) when water content was decreased isosmotically from 65.8 to 58.3% w/w. In cells containing low K and high Na, isosmotic swelling increased $k_K$ from 0.016 ± 0.001 to 0.038 ± 0.004 ($p < 0.005$) and isosmotic shrinking increased $k_{Na}$ from 0.019 ± 0.002 to 0.051 ± 0.005 ($p < 0.005$) suggesting that Li behaves like Na. Osmotic shrinking significantly increased $k_K$ and $k_{Li}$ in cells with high K, and $k_K$ and $k_{Na}$ in cells with high Na. Osmotic swelling did not induce any significant change in the cation ground permeabilities.

The relationship between these observations and known pathways for cation movements across human red cell membranes will be discussed.

Supported by NIH grants GM-25686 and HL-25064.

**T-AM-Po59 ANION COUPLED NA EFFLUX MEDIATED BY THE NA:K PUMP IN HUMAN BLOOD CELLS.** **Steen Dissing and Joseph F. Hoffman. Dept. of Physiology, Yale University, New Haven, CT 06510 .**

**Garrahan and Glynn (J. Physiol. (1967), 192, 159-174) observed a ouabain-sensitive efflux of Na from red blood cells incubated in a choline-buffered medium free of Na and K. This efflux also occurs when $SO_4$ has been substituted for chloride on both side of the membrane and is large enough (as much as 0.7mM/l cells•hr) to be detected if it were electrogenic. However, no ouabain-sensitive change in the membrane potential, monitored fluorometrically with the dye, diS-$C_3$(5), was discernible, indicating that this Na efflux is electroneutral. Since preliminary experiments indicated that protons were not involved in this Na efflux, we measured $^{35}SO_4$ efflux from $SO_4$ loaded DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid) treated red cells suspended at equilibrium in 95 mM $(Tris)_2$ $SO_4$ medium at pH 7.2. The rate constant for $SO_4$ efflux was found to decrease from 0.0047 ± 0.0007 (SD) $hr^{-1}$ to 0.0027 ± 0.0007 $hr^{-1}$ after addition of 0.1 mM ouabain to the medium. This ouabain-sensitive $SO_4$ efflux is completely inhibited when either 5 mM Na or 10 mM K is present in the medium. Ouabain-sensitive efflux is also not apparent in the presence of 15 mM Cl (substituted for $SO_4$). These results indicate that anions accompany the pump efflux of Na that occurs in the absence of external Na and K. The stoichiometry as well as the functional significance of this net salt transport has yet to be determined. (This work was supported by NIH grants HL-09906 and AM-17433.)**

**T-AM-Po60** EFFECTS OF QUININE ON MONOVALENT ANION AND CATION FLUXES IN RAT LIVER MITOCHONDRIA. Richard A. Nakashima and Keith D. Garlid, Medical College of Ohio, Department of Pharmacology, Toledo, Ohio 43699

Reports that quinine inhibits K+ efflux from red blood cells (Reichstein, E. and Rothstein, A., J. Mem. Biol. 59, 57-63, 1981) led us to evaluate its effects on mitochondria. In the presence of EDTA, mitochondria swell spontaneously in KSCN, and this swelling is blocked by 0.5 mM quinine. Quinine also blocks A23187-induced swelling in $KNO_3$. Electrophoretic K+ and Na+ uptake were found to occur in $Mg^{2+}$ and K+ depleted mitochondria, and these uniport pathways were blocked by quinine. Quinine did not affect passive swelling in NaAc or A23187-induced K+ efflux from respiring mitochondria. Quinine did not affect passive swelling in KSCN + valinomycin (Val); KAc + Val; KAc + nigericin (Nig), nor did it affect spontaneous swelling in $NH_4^+$ or trimethylamine (TMA) acetate. Swelling in $KNO_3$ + Val was inhibited about 30% by quinine. Quinine induced rapid swelling in KSCN + Nig, $NH_4SCN$, NaSCN, $NH_4NO_3$, and $TMANO_3$, reactions which require H+ uniport for net salt uptake and swelling. In these systems quinine behaved indistinguishably from CCP. In contrast, quinine had no effect in KAc + Val, while CCP induced rapid swelling in this system.

We conclude from these studies that quinine, like most anesthetics, blocks K+ and Na+ uniport without affecting the endogenous cation/H+ exchangers. Quinine inhibits $NO_3^-$ uniport slightly but not that of $SCN^-$. Quinine has no effect on the actions of ionophores or the non-ionic diffusion of weak acids and bases. Quinine is able to induce H+ uniport under some conditions, but not in $Ac^-$. Present evidence suggests that the uncoupler-like activity of quinine is due to interaction with a membrane component and not to protonophoretic activity. (Supported by USPHS grant GM 24297)

**T-AM-Po61** STEADY-STATE TYRAMINE UPTAKE IN CHROMAFFIN GHOSTS: A BALANCE BETWEEN ELECTROGENIC TRANSPORT AND ELECTRONEUTRAL PERMEATION. Jane Knoth, James O. Peabody, Peter Huettl and David Njus, Department of Biological Sciences, Wayne State University, Detroit MI 48202.

In adrenal medullary chromaffin granules, an $H^+$-translocating ATPase drives uptake of dopamine [2-(3,4-dihydroxyphenyl)ethylamine], but tyramine [2-(4-hydroxyphenyl)ethylamine] is only slightly accumulated. Our studies suggest that tyramine is transported about as fast as dopamine but that its greater lipophilicity allows it to leak back out across the granule membrane. Amine transport into chromaffin-granule membrane vesicles (ghosts) occurs via an electrogenic exchange of 2 $H^+$ for one protonated amine. This transport is mediated by a saturable, reserpine-sensitive translocator. By contrast, amine permeation is unsaturable and reserpine-insensitive and occurs because the unprotonated form of the amine crosses the membrane. The rate of transport is $(\frac{V_{max}C_{out}}{K_m + C_{out}})(1 - \frac{C_{in}}{C_{inT}})$ where $C_{in}$ and $C_{out}$ are internal and external amine concentrations and $C_{inT}$ is the equilibrium internal concentration. The rate of permeation is $V_i(1-C_{in}/C_{inP})$ where $V_i$ is the initial rate of permeation and $C_{inP}$ is the equilibrium internal concentration. The equilibrium concentration for transport ($C_{inT}$) is much higher than that for permeation ($C_{inP}$). At steady-state uptake will lie between $C_{inT}$ and $C_{inP}$ depending on the relative values of $V_{max}$ and $V_i$. By measuring $V_i$ and steady-state concentrations, $V_{max}$ can be estimated. Tyramine and dopamine have comparable values of $V_{max}$ but tyramine's $V_i$ is ten times that of dopamine. Thus, the two aromatic hydroxyls present in the catecholamines are not both required for translocator-mediated uptake. They do, however, limit catecholamine loss through unmediated permeation. Tyramine is a suspected neurotransmitter, but its intrinsic lipophilicity seems to prohibit storage at high concentrations (supported by the Michigan Heart Association and NSF Grant No. BNS-7904752)

**T-AM-Po62** THE CONTRIBUTION OF SURFACE POTENTIAL TO $NA^+$ ENTRY IN FROG SKIN EPITHELIUM. J. Reyes, R. Latorre, and D.J. Benos, Department of Physiology and Biophysics, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.

The effects which alterations in the surface potential of the apical membrane of *in vitro* *Rana catesbeiana* abdominal skin have upon $Na^+$ entry were examined. Apical membrane surface potential was changed in two ways: first, by changing the ionic strength of the external bathing solution and second, by adding various concentrations of $UO_2^{++}$ to the external solution. We found that changes in external ionic strength have little effect upon the rate of $Na^+$ transport. Uranyl ion (2.5 mM) induces a +145 mV or +60 mV change in the surface potential of phosphatidylserine or phosphatidylcholine monolayers, respectively. However, maximal $UO_2^{+2}$ concentrations (10mM) inhibit amiloride-sensitive short circuit current ($I_{sc}$) by only 20% in bullfrog skin, and stimulate $I_{sc}$ by 40% in *R. temporaria* skin. From a steady-state electrodiffusion model in which alterations in apical membrane surface potential are taken into account, a change in surface potential of +60 to +145 mV should inhibit $I_{sc}$ by 85-100%. We conclude that the surface charge density in the neighborhood of the apical membrane $Na^+$ entry site is small (<1e-/600Å$^2$), and that $Na^+$ entry is not affected by changes in membrane surface potential. Supported by N.I.H. Grants AM-25886 and GM-25277.

**T-AM-Po63** DURATION AND MAGNITUDE OF NEGATIVE MEMBRANE POTENTIALS IN RENAL BRUSH BORDER MEMBRANES. Shirley Hilden, Laboratory of Molecular Aging, National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore City Hospitals, Baltimore, MD 21224.

The potential sensitive dye, diS-$C_3$-5, was used to monitor membrane potential changes in rabbit renal brush border membranes. The $NO_3^-$ diffusion potential decayed faster than the valinomycin induced $K^+$ diffusion potential. Results suggested that any potential will decay quickly when the concentration gradients will decay by net movement via diffusion into a smaller compartment such as the intravesicular vesicular volume of brush border membrane vesicles. Since no potential developed when $K^+$ or $Na^+$ gradients were imposed across the membrane, $Na^+$ and $K^+$ permeabilities are apparently low. The magnitude of potentials developed with anion gradients suggests the following permeability sequence: $NO_3^- > Cl^- > H_2PO_4^- >$ gluconate.

**T-AM-Po64** IS CALCIUM-INDUCED CALCIUM RELEASE A GATED CALCIUM TRANSPORT? S. Tsuyoshi Ohnishi
Dept. of Anesthesiology and Dept. of Biological Chemistry, Hahnemann Medical College Philadelphia, Pennsylvania 19102

At low $Mg^{2+}$ concentrations the sarcoplasmic reticulum (SR) can release calcium when the cytoplasmic concentration of $Ca^{2+}$ is increased to about 5 $\mu$M. This phenomenon has been extensively studied with skinned muscle fiber and is known as calcium-induced calcium release (abbreviated as Ca-ICR). For biochemical and biophysical measurements as well as kinetic studies, the use of fragmented SR (FSR) is more desirable than skinned muscle preparations. We have developed a system in which an elevation of approximately 5 $\mu$M $Ca^{2+}$ can effectively induce Ca-ICR from FSR. Results suggest that Ca-ICR takes place through gated calcium channels which may be separated from the Ca-pump itself. The gate opens at approximately 5 to 20 $\mu$M external $[Ca^{2+}]$ and closes again at approximately 40 uM $[Ca^{2+}]$. The addition of caffeine increases the affinity of $Ca^{2+}$ to the high-affinity binding site of the FSR membrane but does not change the permeability of the channel. The $Mg^{2+}$ decreases $Ca^{2+}$ affinity to the binding site and also suppresses the calcium permeability. Ca-ICR demonstrates a positive feedback phenomenon in which the increase of the $Ca^{2+}$ permeability is caused by an increase in the external $[Ca^{2+}]$. The effect of the nucleotide is not to change the affinity of $Ca^{2+}$ to the binding site, but to increase the $Ca^{2+}$ permeability of the channel.

References: (1) Ohnishi, S.T., J. Biochemistry 86: 1147 (1979)
(2) Ohnishi, S.T., in "The Mechanism of Gated Calcium Transport Across Biological Membranes", Academic Press, New York (1981). Eds. S.T. Ohnishi and M. Endo

**T-AM-Po65** CALCIUM TRANSPORT IN ISOLATED CARDIAC CELLS: EFFECTS OF $Na^+$ AND $K^+$. Michel Désilets and Magda Horackova (Intr. by A.Y.K. Wong), Dalhousie University, Halifax, N.S., Canada.

Using $^{45}Ca$ flux determinations, we studied the effects of extracellular cations on calcium transport in the enzymatically dissociated myocytes from adult rat hearts. The morphological and electrophysiological integrity of these cells was confirmed by electron microscopy and membrane potential measurements. The results from the $^{45}Ca$ fluxes studies showed that the effects of $[Na]_o$ and $[K]_o$ were similar to these observed in the intact tissue: i) lowering $[Na]_o$ increased transiently $^{45}Ca$ uptake by stimulating Ca influx and inhibiting Ca efflux; ii) $[K]_o$ = 60 mM increased both Ca influx and Ca efflux. Compartmental analysis of the $^{45}Ca$ uptake experiments suggest that the effects described above are localized in two distinct compartments. The content of the fast compartment (time constant of 0.5 min) is enhanced by lowering $[Na]_o$ as well as by increasing $[K]_o$. The slow compartment (time constant of 24 min), which represents 70% of the total exchangeable Ca, is stimulated by $[K]_o$ but insensitive to $[Na]_o$. Further, our data indicate that caffeine (5mM) inhibits the Ca uptake by the slow compartment without affecting the fast, sodium-dependent one. Our data indicate a presence of two different mechanisms involved in the $Ca_i$ regulation in the isolated myocytes: i) a transmembrane movement of Ca controlled, at least partially, by voltage-dependent Na-Ca exchange mechanism and ii) the sarcoplasmic reticulum which seems to be also directly affected by $[K]_o$.

(Supported by MRC of Canada and by Nova Scotia Heart Foundation).

T-AM-Po66 THE RATE COEFFICIENT OF SODIUM EFFLUX FROM SQUID GIANT AXONS. George R. Kracke and Paul De Weer, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

The rate coefficient of $^{22}Na$ efflux from Na-depleted (≃400-500 μm) axons into normal-Na seawater increased in 3-4 h from 0.003/min to a constant value of about 0.007/min, and that from normal-Na or Na-loaded axons into Na-free seawater decreased in 4-5 h from about 0.008/min to 0.003/min, suggesting that Na efflux from squid giant axon is a nonlinear function of internal [Na]. When Na-depleted axons with steady rate coefficients were injected with NaCl, their rate coefficient into Na-free seawater rose sharply, passed through a maximum, and then decreased to a steady level over a period of 6-7 h. By back addition of the counts leaving the axon, and from a knowledge of the specific activity of $^{22}Na$ at the time of injection of the NaCl, the Na efflux rate can be calculated as a function of internal [Na]. These calculations yield a sigmoid relationship between Na efflux (not corrected for ouabain-insensitive flux) and internal [Na], with Hill coefficients ranging from 1.4 to 2.1 (n=4). This nonlinear dependence was confirmed in internally dialyzed axons where Na efflux was directly measured as a function of internal [Na]. The dialysate contained 2 mM EGTA, and efflux was measured in 40 mM-Na seawater to reduce or eliminate exchange fluxes. Ouabain-sensitive sodium efflux showed a sigmoid dependence on internal [Na], with Hill coefficient 1.8 ± 0.3, Km 27 ± 5 mM, and maximum efflux 75 ± 8 $pmol/cm^2.sec$ (n=6). We conclude that the rate of Na efflux from squid giant axon is an S-shaped function of internal Na, similar to that found for skeletal muscle and red blood cells.
(Supported by NIH grants HL 07275 and NS 11223, and the Grass Foundation).

**T-AM-Po67** A PHOTOSYNTHETIC PHOTOELECTROCHEMICAL CELL, R.L. Pan, R. Bhardwaj, D. Sanderson, I.J. Fan and E.L. Gross, Dept. of Biochemistry, The Ohio State University, Columbus, Ohio 43210

We have developed a photosynthetic photoelectrical cell using both the photoreduction of FMN and photosynthetic electron transport of Photosystem I (Bhardwaj et al. Nature 289:5796 (1981)). Photosystem I (PSI) particles isolated according to the method of Shiozawa et al. (Arch. Biochem. Biophys. 165:388 (1974)) were precipitated on a Metricel filter which was placed between two compartments containing the electron donor couple ($K_4Fe(CN)_6$)/($K_3Fe(CN)_6$) and the electron acceptor (FMN) respectively. A maximum photopower of 1.95 mW was observed when the FMN side was illuminated. The open circuit voltage and the short circuit current were 745 mV and 15 mA. The maximum power conversion efficiency using Pt electrodes was 1.0% in white and 0.16% in red (>600nm) light. The photopower consisted of two parts: the photosynthetic reduction of FMN using $K_4Fe(CN)_6$ as the electron donor and a photochemical part due to the photoreduction of FMN using Tris, Tricine or EDTA. When active PSI particles were used, both processes were functional. The action spectrum had a peak in the red (660 and 690 nm) due to chlorophyll as well as in the blue (400 and 470 nm) due to FMN. When the PSI particles were killed with TCA, only the FMN contribution remained. Substituting reticulated vitreous carbon electrodes for the Pt electrodes increased the power conversion efficiency from 1.0 to 3.8%. Inorganic semiconductors such as CdS can act as light-harvesting agents for the photoreduction of FMN or methyl viologen.

**T-AM-Po68** EPR MEASUREMENTS OF Mn(II) RELEASED FROM SPINACH CHLOROPLAST MEMBRANES BY CHEMICAL AND HEAT TREATMENT. Gary W. Brudvig, David B. Goodin, and Kenneth Sauer, Department of Chemistry and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Mild heat, Tris or $NH_2OH$ treatment of chloroplasts eliminates $O_2$ evolution with release of Mn(II) from the thylakoid membrane. The released Mn is found to bind moderately tightly to the thylakoid membrane and the bound Mn(II) exhibits an unusual EPR signal near zero-field at X-band. The bound Mn(II) EPR signal from chloroplasts is nearly identical to that from Mn(II) bound to bovine serum albumin (BSA). Mn(II)-BSA is used as a standard to quantitate the Mn(II) released from chloroplasts. It is found that most of the Mn released by chemical or heat treatment (3-7 Mn/400 chl) can be accounted for by the bound Mn(II) EPR signal. Less than 1 Mn/400 chl remains as aqueous Mn(II) after release.

(Supported by DOE under Contract No. W-7405-ENG-48 and NFS Grant PCM 79-11251).

**T-AM-Po69** CHARACTERIZATION OF THE LIGHT ANTENNA OF THE PHOTOTACTIC ALGA CHLAMYDOMONAS. Kenneth W. Foster. Mount Sinai Medical Center, CUNY, New York, N.Y. 10029.

Chlamydomonas is a unicellular flagellated green alga able to swim directly toward a source of light. A light detector with a limited field of view, i.e. narrow directivity, and pointing normal to the cell path scans the light environment as the cell rotates. The signal from this scan controls the flagella in such a way that a source of light can be tracked with precision. This light antenna or eye consists of layers of alternating refractive index, each one-quarter wavelength in optical path, beginning one-quarter wave behind the receptor layer (seen by electron microscopy to be in the plasmamembrane) and thus focuses light on the receptor by constructive interference.

The quarter-wave stack optimizes two important antenna properties. The wavelength sensitivity is matched to the light environment without interfering with the sensitivity of other cells. The directivity is matched to the distribution of light providing the best signal possible. The micron size antenna has been measured to be maximally reflective between 500 and 580 nm. Given the sensitivity of the rhodopsin-like receptor chromophore (single 500 nm peak) and the stacks, the system appears designed to operate within the narrow wavelength band that is maximally transparent for the algae. Use of a pigment screen would have destroyed this transparency and reduced the degree of directivity for a membrane surface receptor. The narrowed directivity closely matches the light distribution found on a cloudy day or in the presence of significant light scattering.

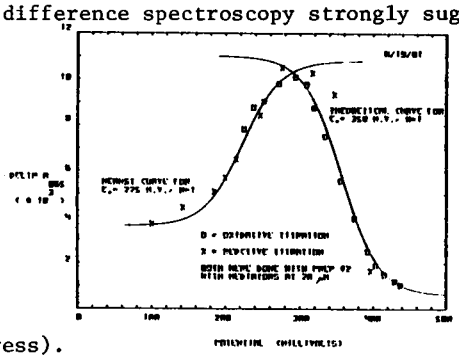
The quarter-wave stack antenna has optimized the system for phototaxis underneath a canopy of green algae. In the absence of such a canopy of cells the system will still function by virtue of its chromophore sensitivity and eyespot absorption below 500 nm.

**T-AM-Po70** NEW RAPID PROCEDURE FOR ISOLATING PHOTOSYNTHETIC REACTION CENTERS USING CYTOCHROME c AFFINITY CHROMATOGRAPHY. Gary W. Brudvig, Stephen T. Worland, and Kenneth Sauer. Department of Chemistry, and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Horse heart cytochrome c linked to Sepharose 4B is used to purify reaction centers from Rps. sphaeroides R-26 and Photosystem I (PSI) reaction centers from spinach chloroplasts. The procedure allows 90-95% recovery of the bacterial and 40-50% recovery of the PSI reaction centers from detergent solubilized membranes and requires only 6-8 hours to obtain purified reaction centers. The PSI reaction centers have a chl:P700 ratio of 20-30. The bacterial reaction centers have OD (280 nm) OD/(802 nm) = 1.6. SDS-PAGE reveals that the bacterial reaction centers contain three polypeptides with apparent molecular weights of 21, 24, and 28 kD as previously reported (Clayton, R.K. and Wang, R.T., Meth. Enz. 23, 696). The only other significant bands in the SDS gel of purified bacterial reaction centers migrated with apparent molecular weights of 45 and 55 kD and are probably dimers of two of the reaction centers.
(Supported by Dept. of Energy under Contract No. W-7405-ENG-48 and by NSF Grant PCM 79-11251)

**T-AM-Po71** KINETICS AND THERMODYNAMICS OF THE PRIMARY PHOTOCHEMISTRY OF THE GREEN NON-SULFUR PHOTOSYNTHETIC BACTERIUM CHLOROFLEXUS AURANTIACUS.
B. D. BRUCE*, R. C. FULLER*, R. E. BLANKENSHIP. CHEMISTRY DEPT., AMHERST COLLEGE AND BIOCHEMISTRY DEPT.*, UNIV. OF MASSACHUSETTS, AMHERST, MASS.

Recently, a cytoplasmic membrane preparation from the green photosynthetic bacteria, Chloroflexus aurantiacus, devoid of antenna bacteriochlorophyll c yet retaining photochemical activity has been described.(1) Single flash induced light minus dark difference spectroscopy strongly suggests a primary donor ($P_{865}$) which is much more similar to the $P_{870}$ bacteriochlorophyll a dimer found in the purple bacteria than to $P_{840}$ found in the other green bacteria. Redox potentiometry of $P_{865}$ ($E_m$ ~ 360 mV) and acceptor (E ≅ 0 mV) suggests that thermodynamically Chloroflexus is different from either the purple or green bacteria. The preparation also contains a c-type cytochrome ($E_m$ = 220 mV) which is closely coupled to the reaction center as indicated by its ability to re-reduce photoxidized $P_{865}$. The photochemistry of Chloroflexus appears to be distinct from other photosynthetic bacteria and may occupy an important role in photosynthetic evolution.



(1) R. G. Feick, M. Fitzpatrick & R.C. Fuller, J. Bact. (in press).

**T-AM-Po72** EFFECTS OF MONOVALENT CATIONS ON THE KINETIC PARAMETERS OF PROTON TRANSPORT IN RECONSTITUTED PURPLE MEMBRANE. Shu-I Tu,[1] Hiroshi Okazaki,[2] Fausto Ramirez, [2] and Howard Hutchinson.[1]
[1] Eastern Regional Research Center, ARS, USDA, 600 East Mermaid Lane, Philadelphia, PA 19118, and
[2] Department of Chemistry, SUNY at Stony Brook, Stony Brook, New York 11794.

The effects of substituting $K^+$ by $Li^+$, $Na^+$, $Cs^+$, and $Rb^+$ in the assay medium on the kinetics of light-induced proton transport of reconstituted purple membrane were analyzed by a previously published scheme (Tu et al., Biochem. Biophys. Res. Comm. 99, 584-590 1981). Significant changes were observed when $K^+$ was replaced by $Na^+$. Although the substitution of $K^+$ by $Na^+$ causes a gradual increase of initial proton pumping rate ($R_O$), the leak rate constants of the light stage ($k_L$) and in the dark ($k_D$) remain unchanged. For reconstituted purple membrane, rate constant $k_L$ is related to $k_D$ and $R_O$ by an equation $k_L = k_D + mR_O$, in which "m" may be regarded as a regulatory constant indicating the linkage between proton pumping ($R_O$) and light-triggered leak pathway ($k_L - k_D$). Thus, the results suggest the response of the leak path to the pumping process through the linkage may be inhibited by the presence of $Na^+$.

T-AM-Po73 RELATIONSHIP OF ELECTRON TRANSPORT RATE AND THE LIGHT DEPENDENT PROTON LEAKAGE THROUGH $CF_0 \cdot CF_1$ COMPLEX IN SPINACH CHLOROPLASTS. Yee-Kin Ho and Jui H. Wang, Bioenergetics Laboratory, Acheson Hall, SUNY at Buffalo, Buffalo, New York 14214.

The basal electron transport rate (from $H_2O$ to Ferricyanide) and the light dependent proton leakage through $CF_0 \cdot CF_1$ complex (Ho, et al. (1979) BBA 547, 149) in chloroplasts were found to have the same pH-dependence which exhibits a high rate at pH 8.0 and a lower rate at pH 6.4. Specific modification on $CF_0 \cdot CF_1$ by dicyclohexylcarbodiimide (DCCD) and orthophenylenedimaleimide (OPDM) show its effect only at pH 8.0 but not at pH 6.4 —— DCCD removes the pH-dependence by lowering the pH 8.0 rate to the pH 6.4 level and OPDM increases the rates at pH 8.0 approximately two-fold. Hydrophobic pyridine homologues such as n-butylpyridine and uncouplers such as FCCP which are known to de-energize the thylakoid membrane and eliminate the light dependent proton leakage through $CF_0 \cdot CF_1$, were found to remove the pH-dependence of the electron transport. Glutaraldehyde fixation which is known to eliminate the light dependent proton leakage was found also to remove the pH-dependence of the rate of electron transport. The pH-dependence of basal electron transport rate in chloroplasts may be related to the light-dependent proton leakage through $CF_0 \cdot CF_1$. The uncoupler FCCP which removes the proton gradient completely was found to have no effect on the electron transport rate of glutaraldehyde-fixed chloroplasts. These results suggest that the regulation of both electron transport chain and the $CF_0 \cdot CF_1$ complex may involve membrane conformational change triggered by membrane potential generated during energization of the thylakoid membrane.

T-AM-Po74 BACTERIOCHLOROPHYLL C DERIVATIVES: POSSIBLE TRANSIENT ELECTRON ACCEPTORS IN GREEN BACTERIA. I. Fujita, A. Forman, L. K. Hanson, K. M. Smith and J. Fajer, Brookhaven National Laboratory, Upton, New York 11973.

Green bacteria appear to straddle green plants and purple bacteria on an evolutionary scale, and contain electron transport components of both classes of organisms. For example, iron-sulfur proteins as well as bacteriochlorophyll a (BChl) have recently been shown to act as early electron acceptors in the primary photochemistry of Prosthecochloris aestuarii (Swarthoff et al. FEBS Letters 131, 331 (1981)). The initial electron acceptor has not been identified, however. The low oxidation potential (+0.24V) of P840, the primary donor of green bacteria, implies that its singlet excited state (~1.46eV) is energetically capable of reducing a primary electron acceptor with a very negative potential. In vitro studies of BChl c derivatives (which are reported to be present in the reaction center) suggest that these chromophores are attractive candidates for the primary acceptor. In vitro reduction potentials for BChl c and bacteriopheophytin c (BPheo), $E_{1/2} = -1.0$ and $-0.8$ V, respectively, indicate that their anions could reduce the subsequent components in the electron transport chain. The optical spectra of the anions resemble those of Chl $a^-$ and Pheo $a^-$, and thus distinguish them from BChl $a^-$ or BPheo $a^-$. BChl $c^-$ and BPheo $c^-$ also exhibit characteristic ENDOR signatures which clearly differentiate them from anions of BChl a derivatives. A possible scheme for energy transduction in green bacteria would thus involve components of both green plants and purple bacteria: P840 → BChl c deriv. → BChl a → [Fe-S]. (Work supported by the Division of Chemical Sciences of the U.S. Department of Energy.)

T-AM-Po75 MOLECULAR HYDROGEN PRODUCTION PHOTOSENSITIZED BY PORPHYRINS: A MODEL FOR THE ORIGIN OF PHOTOSYNTHESIS Janet A. Mercer-Smith and David Mauzerall, The Rockefeller University, 1230 York Avenue, New York, New York 10021

Since life originated when the earth's atmosphere lacked oxygen, the oxidation of the prevalent reduced organic compounds by photoexcited porphyrins with the concomitant emission of molecular hydrogen may have been a precursor of modern photosynthesis. This form of proto-photosynthesis could have established the free energy gradient necessary for life in a reducing atmosphere. If the biosynthetic pathway to chlorophyll recapitulates the evolutionary history of photosynthesis, then porphyrins formed along this biosynthetic pathway (biogenetic porphyrins) served an early photosynthetic function. We report the oxidation of organic electron donors with attendant formation of molecular hydrogen photosensitized by biogenetic porphyrins in aqueous anaerobic solution in the presence of colloidal platinum. The reaction becomes cyclic and hydrogen gas is produced at a constant rate in the presence of methyl viologen, a one-electron carrier. We have prepared two photoactive compounds which serve as models for the evolving biological system for photosynthesis in which reactants are held in close proximity: (1) a coproporphyrin-polyvinyl alcohol-platinum polymer and (2) a compound composed of platinum metal complexed to substituents on the porphyrin periphery. When these model compounds are used in the photoproduction of molecular hydrogen, the necessity of the methyl viologen electron shuttle is eliminated. The evidence indicates that porphyrin radical intermediates, rather than hydroporphyrins, are active in the formation of hydrogen gas.

This work was supported under NSF grants PCM77-0912 and PCM80-1145.

**T-AM-Po76** THE CHEMICAL MODIFICATION OF PLASTOCYANIN. by: S.P. Berg* L.M. Rellick† K.O. Burkey† and E.L. Gross† *The Department of Biological Sciences, University of Denver, Denver, CO, 80208 and †The Biochemistry Department, The Ohio State University, Columbus, Ohio, 43210.

We have used chemical modification to study the redox properties of Plastocyanin (PC) and its ability to donate electrons to P700+. We react PC with ethylenediamine (EDA) in the presence of a water soluble carbodiimide. This converts a negatively charged carboxyl to a positively charged group. The reaction produces four species of modified PC containing 2.1, 3.2, 4.1, and 6.3 molecules of EDA respectively. All four forms show a +40mV shift in midpoint potential (+367 to +405mV) whereas the apparent Km for interaction with P700+ decreases with the increasing extent of modification. The minimally modified forms are labeled in residues 41-45, and the more extensively modified forms are labeled in residues 59-62. 1H-NMR of reduced PC shows changes in tyr-80 and 83 upon modification. Reduction of the protein increased the magnitude of the near UV circular dichroism spectrum. Chemical modification altered the near UV circular dichroism in both the oxidized and reduced forms of PC. EPR measurements show a decrease in the hyperfine coupling constant upon addition of NaCl but no change upon covalent modification.

**T-AM-Po77** PHOTOSYNTHETIC Q-LOOPS AND PHOSPHORYLATION. John Whitmarsh and Thomas Graan, Department of Botany. University of Illinois. USDA/ARS. Urbana, Illinois 61801. U.S.A.

In chloroplasts illuminated by continuous light the transport of electrons from durohydroquinone ($DQH_2$) to methyl viologen (MV) has been shown to be driven by photosystem I and is coupled to ATP synthesis (Izawa, S. and R. L. Pan (1978) BBRC 83:1171-1177). The reaction is inhibited by DBMIB indicating that the pathway of electrons includes the rate-limiting step (plastohydroquinone oxidation). We have investigated this reaction in spinach chloroplasts using single turnover actinic flashes. Spectroscopic measurements at 515 nm reveal a rapid absorbance increase followed by a slow phase. The absorbance change at 515 nm is due primarily to the electrochromic effect of a transmembrane electric field. The slow phase is stable and under some conditions represents as much as 50% of the total electrochromic effect. The rate of the slow phase increase correlates wth cytochrome f reduction, is sensitive to uncouplers, flash frequency and the inhibitor UHDBT. In previous studies the slow phase has been inferred to be due to a Q-loop reaction in which plastoquinone is involved in a transmembrane cycle resulting in the translocation of an additional $H^+$ for each electron passing through the chain. One consequence of an operating Q-loop would be additional ATP synthesis over that of a linear scheme. We have measured the synthesis of ATP in the reaction $DQH_2$ to MV using single turnover flashes. Preliminary measurements indicate a $P/e_2$ of $0.70 \pm 0.05$. In the same chloroplasts the $P/e_2$ for $H_2O$ to MV is $0.95 \pm 0.05$. In the absence of a Q-loop the $P/e_2$ ratio of the reaction $DQH_2$ to MV is expected to be half that of $H_2O$ to MV. Our results show this ratio to be substantially greater than one-half. One interpretation of these data is that the additional ATP synthesized is due to a Q-loop in photosynthetic electron transport.

**T-AM-Po78** ORIENTATION OF MEMBRANE-BOUND CHLOROPLAST CYTOCHROMES. Mark S. Crowder, Roger C. Prince,* and Alan Bearden, Department of Biophysics & Medical Physics, University of California, Berkeley, California, 94720.

Broken spinach chloroplast membranes oriented by partial dehydration on Mylar were examined by EPR spectroscopy at 15K. Dark-adapted, ferricyanide-treated membranes show oriented EPR signals characteristic of oxidized cytochromes. The $g_z$ 3.5 signal of cytochrome f is strongly oriented 65° from the membrane plane in contrast to previous results (1). Additional "high-spin" signals at g 5.65 and g 6.2 are oriented at 60° from the membrane plane; and are assigned to cytochrome $b_6$. Dark-adapted, untreated membranes show signals at g 1.73 and g 2.3 with strong orientations in the membrane plane (2), the orientation expected for the low-potential form of cytochrome b-559. A light-induced signal at 15K with $g_x$ 2.15 is strongly oriented in the membrane plane and is ascribed to high potential cytochrome b-559. The only low temperature EPR signal found not to be oriented was the signal from oxidized plastocyanin.

1. Bergström, N. H. J. and Vänngård, T., Proc. Fifth Int. Congr. Photosyn., Halkidki, GREECE, 1980.
2. Prince, R. C., Crowder, M. S., and Bearden, A., Biochim. Biophys. Acta 592, 323-337 (1980).
*Dept. of Biochem. & Biophys., Univ. of Pennsylvania, Philadelphia, PA 19104.
Research support: Biophysics Program, NSF (PCM 78-22245), Dept. of Energy, LBL.

**T-AM-Po79** Photopotentials in a Liposome-Planar Bilayer Lipid Membrane System Containing Thylakoid Membrane Fragments. J. R. Lopez and H. Ti Tien, Department of Biophysics, Michigan State University East Lansing, MI 48824.

Photopotentials have been measured with conventional electrodes across a planar bilayer lipid membrane (BLM). To one side of the BLM liposomes made with egg lecithin and thylakoid membrane fragments were added. The BLM consisted of a mixture of phospholipids in n-octane. The liposomes were formed by sonicating a mixture of egg lecithin and chloroplasts isolated from spinach leaves. For the potentials to be observed, a divalent ion ($Ca^{+2}$ or $Mg^{+2}$) and a carrier of reducing equivalents, e.g. phenazine methasulfate (PMS) and/or Vitamin $K_3$ have to be present in the side containing the liposomes. The result suggests that association of both BLM and liposomal membrane resulting in electrical contact between the two takes place. The polarity of the photoresponse indicates that the liposomes are charging positive inside. The photoresponses exhibit two components, a fast one and a slow one. The faster component is larger in the presence of only PMS than in the presence of only Vit. $K_3$. This may be due to the difference in location where each of these agents interact with the reaction centers of plants. The fast component of the response is attributed to the initial process of charge separation in the pigmented membrane and the slower component is thought to be due to protons moving inside the liposomes and across the BLM into the liposome free side. The action spectrum of the photoresponse follows the absorption spectrum of the chlorophyll pigmentsin the liposome suspension. Since PMS and Vit. $K_3$ are known to interact photochemically with the photosystem I reaction center of plants, the observed photoresponses are taken as evidence that a potential diff. developes across the thylakoid membranes of chloroplasts under illumination. (NIH grant GM-14911)

**T-AM-Po80** ELECTRON TRANSFER IN PHOTOSYNTHESIS: ROLE OF VIBRATIONAL RELAXATION. William Bialek and Robert F. Goldstein, Department of Biophysics & Medical Physics, University of California, Berkeley, California 94720.

Current theories of electron transfer are all based on essentially the same model describing two electronic states coupled to one or more intramolecular or solvent vibrational modes (1,2). Reaction rates have generally been calculated from this model by applying perturbation theory to the electronic matrix elements and assuming rapid vibrational relaxation. The resulting expressions for the rate constant do not explicitly contain the vibrational relaxation rate. In this paper we show that rigorous application of perturbation theory results in a rate constant which does depend on the vibrational relaxation rate, even for rapid relaxation. Approaches not involving perturbation treatment of matrix elements will be discussed. Implications for the analysis of photosynthetic electron transfer and relations to a charge-transfer band experiment (3) will be presented.

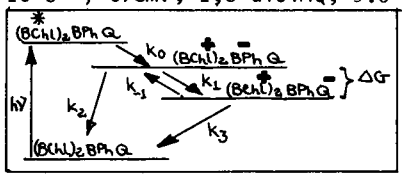
1. De Vault, D., Quart. Rev. Biophys. 13, 387 (1980).
2. Levich, V. G., Adv. Electrochem. & Electrochem. Eng. 4, 249 (1966).
3. Goldstein, R. F. & Bearden, A. (Abstract this meeting).
(Research supported by the Biophysics Program, National Science Foundation (PCM 78-22245) and from the Department of Energy/Lawrence Berkeley Laboratory.

**T-AM-Po81** SUBMICROMOLAR BINDING AND BIPHASIC ELECTRON TRANSFER KINETICS OF CYTOCHROME c AND VESICULAR REACTION CENTERS C.C.Moser, J.M.Pachence, J.K.Blasie, P.L.Dutton. Univ. of Pa., Phila, PA

Reaction centers (RCs) of the photosynthetic bacterium Rps. sphaeroides (R26) solubilized in the detergents LDAO or octyl-glucopyranoside can be reconstituted at a molar ratio of 1/100 with neutral egg lipid (lecithin) to form vesicles of about equal areas of RC and lipid at the vesicle surface and 95% orientation of the RC, cytochrome c (cyt c) reactive side external. Equilibrium redox titrations of equine cyt c in the presence of RC vesicles demonstrate differential binding of oxidized and reduced cyt c to the RC by a 40mV drop in midpoint potential ($E_m$) relative to the unbound cyt c. Redox titrations over a range of protein concentrations indicate dissociation constants ($K_d$) of 0.1 and 0.5μM for oxidized and reduced cyt c, respectively, with a total of 2 binding sites per RC, in agreement with direct measurements (1); such binding seems specific to reconstituted RCs in so far as neutral egg lipid vesicles alone or RCs solubilized in the detergents LDAO or octyl-glucopyranoside do not cause an $E_m$ drop. In comparison, $K_d$=10μM has been reported for both LDAO solubilized RCs and for RC reconstitued via cholate into $2\times10^5$ molar excess neutral lipid (2) while $K_d$=0.4μM has been reported for Triton RCs (3), but here binding gave only 1 cyt c bound per RC and was oxidation state indiscriminate. Flash excited vesicular RCs exhibit biphasic oxidation kinetics of cyt c following two first order rate constants in bound cyt c of $2\times10^4 s^{-1}$ and $1\times10^3 s^{-1}$. $K_d$s for both rates are 0.5μM, in agreement with that value found by redox titrations. Second order slow phase kinetics was not observed. 1. Biphys. J. 25, 55a (1979) 2. Biochem. 19, 3322 (1980) 3. Ibid. 19 (1980) Supported by NSF PCM 09042 and NIH GM 27309.

**T-AM-Po82** VARIATION IN RATES OF ELECTRON TRANSFER IN PHOTOSYNTHETIC REACTION CENTERS WITH THE PRIMARY UBIQUINONE SUBSTITUTED WITH OTHER QUINONES. M.R. Gunner, Y. Liang, D.K. Nagus, R.M. Hochstrasser, P.L. Dutton. Dept.Biochem. and Biophys., U. of Penn., Phila, PA 19104

Rates of electron transfers have been obtained between the bacteriochlorophyll dimer, $(BChl)_2$, bacteripheophytin, (BPh) and quinone (Q) in reaction centers (RCs) from Rps. sphaeroides where the native ubiquinone-10 (UQ) has been replaced by Qs of lower $E_m$s for $Q/Q^-$. Reactions connecting the redox states of RCs are shown in the figure . Rates for $k_0$ ($4\times10^{11}$ $s^{-1}$), $k_2$ ($7\times10^7$ $s^{-1}$) and $k_3$ ($10s^{-1}$) are assumed to be Q independent. Rate $k_1$ was obtained either by observing the rate of decay of $BPh^-$ which occurs at $k_1+k_2$ or from the fraction ($\Phi$) of RCs with a charge separation $\mu$s after the initiating flash ($\Phi=k_1/(k_1+k_2)$). For sufficiently low $E_m$ Qs, $k_1$ can be combined with values for $k_{-1}$, ($k_{-1}$ calculated from the rate of reduction of $(BChl)_2^+$ ($k_{-1}k_1/(k_1+k_2)+k_3$), to obtain the $\Delta G$ for $BPh^-$ reduction of Q. The values for $k_1$, $k_{-1}$ (calculation from $\Phi$ in parenthesis) and $\Delta G$ where it could be calculated are: $UQ_8$ $3\times10^9$ $s^{-1}$ ($>6\times10^8$ $s^{-1}$); tetra methyl benzoQ, $1.4\times10^9$ ($>6\times10^8$ $s^{-1}$); 2,3-methyl napthoQ, $1.2\times10^8$ ($>6\times10^8$ $s^{-1}$); anthraQ (AQ), $8.3\times10^8$ $s^{-1}$ ($6.3\times10^8$ $s^{-1}$) 326mV; 2-ClAQ, $8.4\times10^8$ $s^{-1}$ ($6.3\times10^8$ $s^{-1}$) 378mV; 1,8-diClAQ, $9.5\times10^8$ $s^{-1}$ ($>6\times10^8$ $s^{-1}$) 405mV; 2-ethylAQ, $1.7\times10^8$ $s^{-1}$ ($2.1\times10^8$ $s^{-1}$) 236mV; 2-methylAQ $1.2\times10^8$ $s^{-1}$ ($>6\times10^8$ $s^{-1}$) 238mV; 2,3-methylAQ ($4.9\times10^7$ $s^{-1}$) 184mV and 1-aminoAQ ($1.2\times10^7$ $s^{-1}$) 164mV. Assuming that the relative $E_m$s of $Q/Q^-$ for different Qs is the same in RCs as in vitro, $\Delta G$ can be obtained by extrapolation for Qs where $k_{-1}$ is not observable. When this is done for UQ a $\Delta G$ of 480mV is obtained. NSF. PCM 09042; DOE 10590.




**T-AM-Po83** TIME RESOLVED, RAPID INCREASE IN THE TRANSMEMBRANE POTENTIAL OF H. HALOBIUM CELL ENVELOPE VESICLES. S.L. Helgerson*, Zs. Dancshazy+, W. Stoeckenius*, E. Heinz#. *UCSF, San Francisco, CA 94143, +Institute of Biophysics, Szeged, Hungary, #Cornell Univ. Med.Col., New York, NY 10021.

As it takes many fewer ions to saturate the cellular membrane electric capacity than to produce an equivalent membrane-diffusion potential, an electrogenic $H^+$-pump should initially raise the electrical potential prior to the generation of sizeable ion gradients. It has been postulated that such a rapid rise, provided it is high enough, would make the proton motive force available almost immediately after the onset of pumping (Heinz, in Hydrogen Ion Transport, Elsevier/North-Holland Biomedical Press, 1980). This has been tested with the light-driven $H^+$-pump in H. halobium cell envelope vesicles. The electric membrane potential was measured with msec time resolution by monitoring the M photochemical intermediate of bacteriorhodopsin. An inverse, linear relationship has been found to exist between the decay kinetics of $M_{412}$ and the membrane potential (Dancshazy, Helgerson, and Stoeckenius, in preparation). By applying square pulses of illumination (632 nm) of varying duration, it was shown that the electric potential reaches the predicted magnitude with a half-time of 10-20 msec. This rapid potential rise was strongly suppressed by shunting the potential with gramicidin, and was absent with isolated (non-vesicular) purple membranes under the same conditions. These findings strongly support the above postulate. (Supported by NIH Program Project Grant GM 27057, NIH Grant RO 1 GM 26554-01, and NSF INT. 78-27606).

**T-AM-Po84** EPR STUDIES OF CARBOXYL GROUPS IN SPIN-LABELLED BACTERIORHODOPSIN by Jeffrey M. Herz, Rolf Mehlhorn and Lester Packer, Membrane Bioenergetics Group, Lawrence Berkeley Laboratory, and the Department of Physiology-Anatomy, University of California, Berkeley, CA 94720.

Bacteriorhodopsin (BR) was covalently spin-labelled by reaction with 4-amino-2,2,6,6-tetramethylpiperidino-1-oxy (TEMPAMINE) using EEDQ as a coupling agent. EEDQ is a hydrophobic, highly specific, reagent for the activation and subsequent modification of carboxyl residues and its reaction with BR has been previously characterized (Herz, J.M. and Packer, L., FEBS Lett. 131, 158 (1981)). Modification did not alter the visible absorption maxima and samples retained photocycling activity. Double integration of the first derivative EPR spectra showed that after one hour of reaction with 10 mM EEDQ, 1.2 moles of spin were bound per mole of BR. Spin resonance spectra revealed varying degrees of immobilization of the nitroxide labels, implying that a heterogeneous population of carboxylic side chains had reacted. Progressive treatment of spin-labelled BR with paramagnetic broadening agents eliminated the partially immobilized signal until only a highly immobilized spectrum remained. We infer that surface groups accessible to the quencher are less immobilized than those buried within BR and that appreciable labelling of buried residues occurs. Denaturation of BR with urea, SDS, and heat caused the spins to become more mobile and accessible to quenching whereas increasing the ionic strength with sodium chloride decreased spin mobility and quenching. We conclude that carboxyl spin-labelled BR is a sensitive indicator of conformational states which may be useful for detecting light-induced changes in structure during the photochemical cycle.

Research supported by the Department of Energy and NIH (GM-24273).

**T-AM-Po85** FLEXIBILITY OF BACTERIORHODOPSIN'S C-TERMINAL TAIL. R. Renthal, N. Dawson and P. Horowitz, Div. Earth & Physical Sciences, Univ. of Texas at San Antonio, and Dept. of Biochemistry, Univ. of Texas Health Science Center at San Antonio, San Antonio, Texas 78285

The carboxyl-terminal region of bacteriorhodopsin (BR) is thought to protrude from the surface of the purple membrane (PM). Reaction of PM with dansyl hydrazine (DH) in the presence of water-soluble carbodiimide labels only two regions of BR: the C-terminal tail (residues 232-248) (0.7 mol /mol BR), and a carboxyl group in the sequence 72-118 (0.3 mol/mol). Since the fluorescence from the 72-118 site is over 90% quenched by energy transfer to retinal, virtually all dansyl emission comes from the labeled C-terminal region. We have studied the rotational mobility of the C-terminal tail by fluorescence polarization and proteolysis. Prior to spectroscopy, non-covalently bound DH was removed by several cycles of centrifugation and suspension. The steady-state polarization of DH fluorescence on the tail was 0.24, indicating little local flexibility. Proteolysis of unmodified PM by papain (0.026 BAEE u/ml) at pH 8 was analyzed by gel electrophoresis. The cleavage occurs in two steps. The first order rate constants were: $k_1$ = 0.23 $min^{-1}$, $k_2$ = 0.011 $min^{-1}$. After labeling with DH, the rate of the first step was much slower (0.026 $min^{-1}$), while the second step was unaffected (0.009 $min^{-1}$). During papain cleavage, the polarization decreases from 0.24 to 0.10 in 4 hrs (23°). The rate of decrease, when compared with the cleavage rate, indicates that only the second cleavage releases fluorescence. The results suggest that residues 232-239 are rigidly held at the membrane surface. (Supported by grants from NSF, NIH and the Robert A. Welch Foundation.)

**T-AM-Po86** ACID-BASE EQUILIBRIUM OF THE SCHIFF BASE IN BACTERIORHODOPSIN, S. Druckmann and M. Ottolenghi, Dept. of Physical Chemistry, The Hebrew University, Jerusalem, Israel and A. Pande, J. Pande and R. H. Callender, Dept. of Physics, City College of New York, New York, N.Y. 10031.

Aqueous suspensions of dark-adapted bacteriorhodopsin ($bR_{560}$) in the purple membrane of Halobacterium halobium are exposed to rapid jumps to high pH. Optical and resonance Raman measurements are carried out using flow and stationary methods. Above pH $\simeq$ 11.5 $bR_{560}$ starts to be reversibly converted to a species absorbing at 460 nm ($bR_{460}$) characterized by an unprotonated Schiff base chromophore. Above pH $\sim$ 13.0 bleaching takes place, first reversibly and subsequently irreversibly, to a species absorbing around 365 nm ($bR_{365}$). This process competes with the formation of $bR_{460}$. The pK corresponding to the $bR_{560}+H_2O \longrightarrow bR_{460}+H_3O^+$ equilibrium is determined as $13.3\pm.3$. The values of the corresponding dissociation and association rate constants ($k_d$=0.02 $sec^{-1}$ and $k_a$=3.0 x $10^{11}$ $M^{-1}$ $sec^{-1}$) are in qualitative agreement with the simple relationship $k_d = k_a\ 10^{-pK}$ as observed for homogeneous (diffusion controlled) acid-base equilibria. The results imply that the Schiff base is titrable in the dark and is as accessible to external protons (or to $OH^-$ ions) as a free acid in solution. It is thus directly demonstrated that a light induced pK change of at least nine units takes place during the photocycle of light adapted bR. This conclusion has important implications on the proton pump mechanism.

**T-AM-Po87** ON THE C=N STRETCHING FREQUENCY OF PROTONATED SCHIFF BASES IN VISUAL PIGMENTS, BACTERIORHODOPSIN AND THEIR ANALOGUES. Hiroko Kakitani, Toshiaki Kakitani, Barry Honig and Robert Callender, Dept. of Biochemistry, Columbia University, New York, NY 10032 and Dept. of Physics, City College of the City University of New York, New York, NY 10031.

The Raman line corresponding to the C=N stretching mode of the protonated Schiff base of retinal has been used extensively in visual pigment and bacteriorhodopsin research. In particular, deuterium isotope effects have been used to detect the presence or absence of protonation in a variety of species. However, the actual frequency of the C=N stretch has provided little structural or mechanistic information.

In contrast, the frequency of the intense C=C stretching mode seen im Raman spectra has been widely used and interpreted as a measure of $\pi$ electron delocalization. In general, bond alternation (and hence double bond strength) in polyenes decreases as $\pi$ electron delocalization increases. Increased delocalization is also associated with bathochromic shifts in electronic spectra. Thus an inverse correlation between absorption maxima and C=C stretching frequencies is expected and is, indeed, experimentally observed. Surprisingly, no similar relationship is seen for the C=N stretch even though it too participates in the $\pi$ electron system of the chromophore.

A number of factors contribute to the anomalous behavior of the C=N stretch. These include coupling to bending modes and in particular to the adjacent C-C single bond. This bond is highly sensitive to changes in $\pi$ electron delocalization and acts to reverse the effects expected on the basis of the C=N stretch alone. These and other factors that influence the C=N frequency are discussed. Supported by NSF PCM 81-18058 and NIH EY03142.

**T-AM-Po88** SPECTRAL SHIFTS IN CHLOROPHYLL AND BACTERIOCHLOROPHYLL SYSTEMS INDUCED BY POINT CHARGES. Joseph Eccles, Department of Physiology and Biophysics, U. of Illinois, Urbana, IL 61801; and Barry Honig, Department of Biochemistry, Columbia U., New York, NY 10032.

The in vivo absorption spectra of chlorophyll and bacteriochlorophyll systems are shifted with respect to the spectra of the isolated chromophores. The success of the external point charge model in explaining the in vivo spectra shifts in the retinal Schiff base chromophore of visual pigments leads to the postulate that such a model might also be useful in explaining the shifts in the photosynthetic system. Recent experiments show that small shifts can be induced by placing a charge in the vicinity of a chlorophyll a molecule (R.C. Davis, S.L. Ditson, A.F. Fentiman, and R. M. Pearlstein; J. Am. Chem. Soc., to be published.)

We have treated various chlorophyll and bacteriochlorophyll-like molecules by the CNDO/S method. The spectral properties have been studied both for the isolated molecules and in the presence of external charges. These calculations show large spectral shifts in some cases. The results of these calculations are examined in light of the experimentally known shifts.

**T-AM-Po89** Photoelectric Effect In Bilayer Lipid Membranes Containing Covalently Linked Porphyrin-Carotene Complexes, N. B. Joshi and H. Ti Tien, Department of Biophysics, Michigan State University, East Lansing, MI 48824

In view of the important role played by carotenoids in bacterial and plant photosynthesis, the model pigmented membranes containing carotenoids have been investigated by several workers. However, the exact function of the carotenoids is not yet clear. Our present studies deal with the photoelectric effect in bilayer lipid membranes (BLMs) containing porphyrin-carotene complexes. BLMs were formed in the usual manner separating two aqueous solutions (0.1M sodium acetate buffer pH 5.00). $FeCl_3$ (1 mM) on one side and ascorbic acid (10 mM) on the other side were added after the BLM formation. A large photovoltage 300 mV was observed in BLM containing porphyrin-carotene complex in which porphyrin and carotene molecules are in a folded configuration. It is worth noting that the magnitude of the photoeffect depends on the geometry of the complex and/or the distance between the porphyrin and carotene moieties. The photoeffect was also studied in BLM containing a mixture of porphyrin and carotene, in which the photovoltage increased in the presence of carotenes. The photovoltage with flash light illumination shows no latency in the photoprocesses within the limit of our instrument. The effect of external electric field was also studied. The solution absorption spectra. The results are explained in terms of energy transfer and electron transfer processes. [Supported by a NIH grant GM-14971]

T-AM-Po90 PHOTOCYCLING AND NONCYCLING BACTERIORHODOPSIN CHROMOPHORES UNDERGO DIFFERENT ROTATIONAL DISPLACEMENTS WITHIN THE PURPLE MEMBRANE

Pat Ahl and Richard Cone, Biophysics Dept., The Johns Hopkins Univ., Baltimore, Md. 21218

We examined the transient dichroism properties of suspensions of purple membranes at 400, 570, and 650nm using flash photometry with linearly polarized light. At temperatures over 30°C the dichroism at 570nm decreased with a $t_{\frac{1}{2}}$ of 5-10 msec whereas simultaneously at 400nm the dichroism increased. Near 0°C, when the fragments were suspended in 30% ficoll, the 400nm dichroism decreased ($t_{\frac{1}{2}}$=.2-.3 sec) while the 570nm dichroism increased and the 650nm dichroic ratio exceeded 3.0 . These results cannot be explained by rotational diffusion of the fragments. Moreover the half-times of the dichroism decay at 570nm for temperatures over 30°C were significantly shorter than expected for rotational diffusion of the membrane fragments, which had effective radii of 0.5μ as determined by electron microscopy. Also the the temperature dependence, $Q_{10}$, was 2.6-- too high for rotational diffusion in water. The dependence of the transient dichroism on the measuring light wavelength suggested that different populations of chromophores might undergo different rotational displacements. By considering the simplest possible case, i.e. only two chromophore populations, the photocycling and the noncycling chromophores, we have been able to account for the observed changes in dichroism at all measuring light wavelengths. At temperatures above 30°C, both the cycling and noncyclingly chromophores become rotationaly displaced during the photocycle by as much as 30° from their original postions. During the maximum formation of "M", the cycling chromophores become more rotationally displaced than the noncycling chromophores by about 10-12° ,whereas during the formation of "O", the noncycling chromophores become more rotationally displaced than the cycling chromophores by 5-10°. At temperatures near 0°C, little if any "O" forms and during the maximum formation of "M", the cycling chromophores rotate 17-20° more than the uncycling chromophores. After this point in the cycle both populations continue to rotate slowly at the same rate, approaching complete randomness with respect to their original orientations with half-times of several hundred msec. Since these rotations were eliminated by 4% glutaraldehyde, which crosslinked bacteriorhodpsin, they probably result from rotations of the proteins rather than chromophore rotations within the proteins.

T-AM-Po91 FOURIER TRANSFORM INFRARED DIFFERENCE SPECTROSCOPY OF PURPLE MEMBRANE. Kenneth J. Rothschild, Mark Zagaeski and William A. Cantore. Departments of Physics and Physiology, Boston, MA 02215

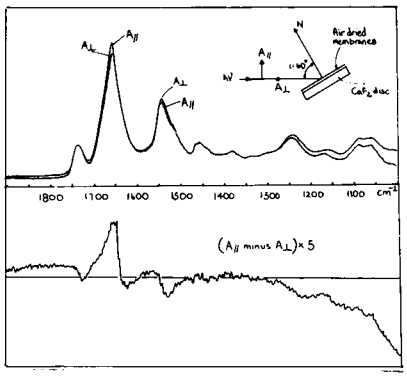
Molecular changes occurring in bacteriorhodopsin can be detected by measuring the difference between Fourier transform infrared spectra measured for dark and illuminated purple membrane films. In contrast to resonance Raman spectroscopy, infrared light does not drive the photocycle and probes all the components of the membrane and not just the retinylidene chromophore. On the basis of isotopic labelling it is established that the largest changes in the difference spectrum are due to shifts in the C=C stretching mode of the retinylidene chromophore. This result is unusual since the chromophore constitutes approximately 1% of the total mass of bacteriorhodopsin. Deuteration of the samples allows identification of the Schiff base linkage to the protein. It is concluded on the basis of this evidence that the Schiff base is protonated in bR570 in agreement with resonance Raman results. Several non-chromophoric vibrations are identified including a carboxylate group mode. This group appears to undergo protonation between the bR570 and M412 state. The absence of large changes in the amide I carbonyl regions rules out large changes in the protein backbone or appreciable changes in the orientation of the alpha-helices. A model more consistent with the FTIR data would involve only local alterations in a small number of residues combined with a large rearrangements of the chromophore.

T-AM-Po92 COMPARISON OF THE ORIENTATION OF THE PRIMARY DONOR TRIPLET IN DIFFERENT PHOTOSYNTHETIC BACTERIA, Brian J. Hales, Karen S. Howard* and Ellen E. Case, Departments of Chemistry and Microbiology*, Louisiana State University, Baton Rouge, LA 70803

ESR spectroscopy has been used successfully to determine the orientation of the principal axes of various paramagnetic proteins in oriented membrane systems We have already investigated the orientation of the photo-induced triplet of the primary donor in the reaction center protein of the photosynthetic bacterium Rhodospirillum rubrum using membrane multilayers (BBA 548,276(1979)). We now extend this study to 12 different photosynthetic bacteria to determine the similarities in the orientation of this key protein. The orientation of the triplet was found to be similar in this group of bacteria where the spectroscopically labeled x- and z- axes are approximately parallel to the membrane plane and the y- axis is parallel to the membrane normal. Each individual bacterium, however, shows its own unique orientation which may differ by 30° from the average of the group. Similarly, the angular standard deviation of the principal axes about their unique orientation varies from species to species. In fact, several bacteria such as the R-26 mutant of Rhodopseudomonas sphaeroides possess a high degree of reaction center order with an angular standard deviation of only 15°. Finally, we have successfully produced oriented multilayers by layering whole cells of bacteria such as R. rubrum which lack internal lamellar membrane structure.

**T-AM-Po93** POLARIZED INFRARED SPECTROSCOPY OF BACTERIAL PHOTOSYNTHETIC REACTION CENTER MEMBRANE MULTILAYERS, E. Nabedryk, D. Tiede, P.L. Dutton, J. Breton, Service Biophysique, C.E.N. Saclay, 91191 Gif-sur-Yvette, France and Dept. Biochem. and Biophys., U. of Penn., Phila.,PA 19104.

The protein structure of Rps. sphaeroides R-26 reaction centers has been examined by polarized infrared(IR) and ultraviolet circular dichroism(UVCD) spectroscopies. Initial analysis of the UVCD spectra of isolated reaction centers (containing LM and H subunits, with 280/800nm absorbance ratio of 1.3) in detergent solution and reconstituted vesicles show the protein to have an α-helix content of 50±10%, with the remainder composed of both random and β-sheet conformations. With reconstituted membrane multilayers oriented on to $CaF_2$ discs, the polarized IR spectra show a small preferential absorption of the amide I band with light polarized parallel to the plane of incidence($A_{//}$), and the corresponding preferred absorption of the amide II band perpendicular to the plane ($A_{\perp}$). Shoulders on the amide I band also suggest slight $A_{\perp}$ preferred absorption by the β-configurations. Pronounced dichroism of the visible absorption bands show the reaction center and membranes to be well-oriented with a mosaic spread ≤25°, as estimated from similar membranes by EPR(1) and diffraction techniques(2). The reaction center protein appears to have a similar composition and orientation as the antenna proteins in chromatophores(3) with the α-helix segments aligned somewhat normal to the membrane. (1)BBA 637(1981)278-290; (2)BBA 635(1981)267-283; (3)BBA 635(1981) 515-524. NSF 79 09042




**T-AM-Po94** MÖSSBAUER STUDIES ON REACTION CENTERS FROM R. SPHAEROIDES R-26 IN STRONG MAGNETIC FIELDS. B. Boso and P. G. Debrunner, Physics Department, University of Illinois at Urbana-Champaign, Urbana, IL 61801 and M. Y. Okamura and G. Feher, Physics Department, University of California at San Diego, La Jolla, CA 92093.

We report high-field (1 T ≤ B ≤ 6.15 T) variable temperature (4.2K ≤ T ≤ 211K) Mössbauer measurements on photosynthetic reaction centers (RCs) isolated from an $^{57}$Fe-enriched culture of R. sphaeroides R-26. Both native (N) and dithionite-reduced (R) RCs were studied. Previous measurements on these samples (B. Boso et al., BBA in press) showed that they contain a single iron species in the high-spin ferrous state, in agreement with magnetic susceptibility data (W. F. Butler et al., Biophys. J. 32, 967-992, 1980). The present results yield further details about the electronic state of the iron. At low temperatures and fields the spectra of N and R differ; the former consists of a sharp quadrupole doublet, while the lines in the latter are magnetically broadened via spin-spin interaction with the semiquinone free radical. In high field the spectra of N and R are similar showing broad doublets at 4.2K, which resolve at higher temperatures. The width of the lower energy line has a minimum near 25K. Analysis of the data shows that (i) the quadrupole tensor is rhombic, (ii) its largest component is positive, (iii) it is rotated with respect to the internal magnetic field $\vec{H}_{int}$, (iv) the three components of $\vec{H}_{int}$ differ widely in size and temperature dependence, and (v) if $\vec{H}_{int}$ is expressed in a spin Hamiltonian model, $\vec{H}_{int} = -\langle\vec{S}\rangle\overleftrightarrow{A}/(g_N\beta_N)$, the magnetic hyperfine tensor $\overleftrightarrow{A}$ is found to be small and anisotropic. We conclude that $\overleftrightarrow{A}$ has a sizable orbital contribution, and that the ligand field of the iron has lower than rhombic symmetry. Supported by Grants from NSF and NIH.

**T-AM-Po95** ON THE ELECTROCHROMIC RESPONSE OF CAROTENOIDS IN PHOTOSYNTHETIC MEMBRANES. Toshiaki Kakitani and Barry Honig, Department of Biochemistry, Columbia University, NY NY 10032 and A.R. Crofts, Department of Biophysics, The University of Illinois, Urbana, IL 61801.

Molecular orbital calculations are carried out on a number of carotenoids in the presence of an external charge and a constant electric field. The external charge is used to represent the strong permanent field which is believed to polarize carotenoids in photosynthetic membranes and thus to account for their linear response to the trans membrane potential. Our calculation shows that the in vitro→in vivo spectral shifts of carotenoids (∿25 nm) can be produced by a charge in close proximity to the molecule. The interaction of the induced dipole moment with a constant field accounts for the observed magnitude of the electrochromic response in photosynthetic bacteria. The existence of a pool of cartenoids that shows significant (∿20 nm) wavelengths shifts but no electrochromic response can be explained by an external charge positioned near the center of the molecule that affects its absorption maximum while inducing essentially no dipole moment. The spectral shift is due to the induction of higher multipoles. These also account for discrepancies that arise when one attempts to account quantitatively for available experimental results on carotenoid band shifts in terms of classical electrochromic theory.
Supported by NSF PCM 81-18088.

**T-AM-Po96** OBSERVATION OF MANGANESE BY X-RAY ABSORPTION SPECTROSCOPY IN CHEMICALLY MODIFIED SPINACH CHLOROPLASTS AND SUPEROXIDE DISMUTASE. D. B. Goodin, J. Fee*, K. Sauer, and M. P. Klein. Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720; *Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109.

The modification of the electronic state of manganese centers in the oxygen evolving complex (OEC) is observed for various conditions of altered function. Complete and reversible inactivation of oxygen evolution by $NH_3$ or tetraphenyl boron does not result in Mn release as observed by EPR. Observation of the manganese electronic environment by X-ray Absorption Edge Spectroscopy (XAES) indicates no direct interaction of these agents with the manganese in the $S_1$ state of the OEC. Irreversible inactivation of the OEC with 1 mM $NH_2OH$ causes the release of only 1 Mn/chl into an aqueous environment and the creation of ∿5 Mn/400 chl in a bound state exhibiting a low field EPR transition at 10°K. This state is observed by XAES as a radically altered absorption edge similar to aqueous $Mn^{+2}$ but with edge features which are significantly broadened. Treatment of active membranes with 0.3% $H_2O_2$ induces a shift of 1.3 eV in the x-ray edge position, with no indication of a significant change in manganese ligand symmetry. The manganese K-edge of native ($Mn^{+3}$) superoxide dismutase from T. Thermophilus is nearly identical in edge shape and position to that in active chloroplasts. Reduction of the SOD with dithionite induces a similar edge shift of 1.5 eV suggesting a structural analog between the two systems. It is proposed that $H_2O_2$ interacts with the OEC in state $S_1$ by acting as an electron donor, reducing $Mn^{+3}$ to $Mn^{+2}$ without its extraction from the OEC.

(Supported by DOE W-7405-ENG-48 and NSF PCM 78-1212)

**T-AM-Po97** ORIENTED PROPERTIES OF THE CHLOROPHYLLS: ELECTRONIC ABSORPTION SPECTROSCOPY OF ORTHORHOMBIC PYROCHLOROPHYLLIDE a - APOMYOGLOBIN SINGLE CRYSTALS. Steven G. Boxer,[a] Atsuo Kuki,[a] Pat O'Hara,[a] Brad Katz,[b] and N. H. Xuong[b]. [a]Department of Chemistry, Stanford University, Stanford, CA 94305; [b]Department of Chemistry, University of California, San Diego, La Jolla CA 92093.

The orientations of the transition dipole moments in chlorophyll (Chl) are among the most useful spectroscopic properties for determining macromolecular architecture in photosynthetic complexes; however, the relationships between these orientations and the Chl molecular geometry are unknown. In order to solve this problem, we have prepared single crystals of the synthetic 1:1 complex between pyrochlorophyllide a and apomyoglobin (apoMb) [S.G. Boxer & K.A. Wright, J. Am. Chem. Soc., 101, 6791 (1979)]. The protein crystallizes readily in the orthorhombic (B) form, space group $P2_12_12_1$, and the unit cell parameters are determined to be within 0.5% of those for native metMb crystals of the same type. These green crystals are highly dichroic, and the strong absorption along the crystallographic a axis in the $Q_y$ band is red-shifted by about 9nm, relative to the corresponding feature in a solution of the protein. Although the crystal structure for native Mb in this space group has not been determined, the direction cosines of the heme normal relative to the crystal axes have been measured. Using these values, an appropriate trigonometric analysis, and the measured polarized single crystal spectra, the orientation of the Chl transition dipole moment for the $Q_y$ transition can be specified relative to the crystal axes. With the completion of the crystal structure, this result will lead directly to the orientations of the optical transition dipole moments relative to the molecular geometry. The effects of vibronic coupling and the protein environment on the absorption properties of Chl will be discussed in detail.

**T-AM-Po98** PHOTODESTRUCTION OF THE RETINYLIDENE CHROMOPHORE OF BACTERIORHODOPSIN BY ULTRAVIOLET LIGHT. Paul K. Wolber and Roberto Bogomolni, Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

Illumination of the bacteriorhodopsin (bR)-containing purple membrane (pm) cast into polyacrylamide gels with c.a. 0.3 $mW/cm^2$ of ultraviolet (UV) light leads to irreversible (i.e. not regenerable with retinal) loss of the absorbance of the retinylidene chromophore at 570 nm. The loss kinetics are first order in time and illuminating intensity, with a $t_{½}$ of c.a. 15 min. The action spectrum of the photochemistry is essentially the absorption spectrum of tryptophan. Replacement of the retinal in pm with retinal $A_2$ shifts the absorption maximum of bR to 600 nm, but changes neither the action spectrum nor the rate of destruction. Difference spectra show a broad increase in absorbance from 250 to 350 nm, with a negative notch having the characteristic position and shape of tryptophan's absorption spectrum. The kinetics are completely insensitive to monovalent cations (up to 1M) and replacement of $H_2O$ by $D_2O$. The rate increases 4x in the pH range 4→1, and decreases 1/3 in exhaustively deoxygenated buffer. Finally, an Arrhenius plot of the observed rates yields an activation energy of 3 kcal/mole for the rate-limiting step. These results indicate that UV light activates tryptophan to destroy the retinylidene chromophore in bR. The presence of an appreciable destruction rate in the absence of $O_2$ shows that photooxidation is not the chief destruction mechanism. The activation energy suggests a simple motion of some group within the protein as the rate-limiting step. We are currently investigating the photoselective properties and chemistry of this process, in order to use the destruction as a probe of the structure of bR. (Supported by NIH: GM 07447 & 27057, and NASA 7151.)

**T-AM-Po99** INFRARED SPECTRUM OF PURPLE MEMBRANE: A CLUE TO A PROTON CONDUCTION MECHANISM? S. Krimm and A. M. Dwivedi, Biophysics Research Division, University of Michigan, Ann Arbor 48109.

The infrared spectrum of dry or wet purple membrane exhibits amide I (C=O stretching) and amide A (NH stretching) frequencies, $\nu(I)$ and $\nu(A)$, that are anomalously high for typical right-handed α-helix conformations, including coiled-coil structures. The amide II (NH in-plane bend) frequency, $\nu(II)$, however, is typical of such structures. We have done normal mode calculations on the α-helix, both for the standard $\alpha_I$ structure as well as the less common $\alpha_{II}$ structure (which, however, has the same n and h). At least four predictions agree with experimental observations, and indicate that the helices in bacteriorhodopsin have the $\alpha_{II}$ structure: 1) $\nu(I)$ and $\nu(A)$ are higher in $\alpha_{II}$ than in $\alpha_I$; 2) $\nu(I)[A] - \nu(I)[E_1]$ is larger for $\alpha_{II}$ than for $\alpha_I$; 3) $\nu(II)[E_1]$ is about the same for $\alpha_{II}$ and $\alpha_I$; and 4) the intensity ratio $\nu(I)[E_1]/\nu(II)[E_1]$ is larger for $\alpha_{II}$ than for $\alpha_I$. The $\alpha_{II}$ structure, in which the plane of the peptide group is tilted with respect to the helix axis with C=O projecting outward, may result from additional hydrogen bond stabilization between side chain serine and threonine OH groups and main chain peptide groups. The shorter distance between adjacent amide hydrogens in the $\alpha_{II}$ structure raises the possibility that the helix of NH hydrogens, rather than side chain residues, may constitute the "proton wire" for translocating protons across the membrane. The vectorial nature of the α-helix backbone, combined with the net in→out electric dipole moment of bacteriorhodopsin, suggests that such a mechanism merits serious consideration. This research was supported by NSF grants PCM79-21652 and DMR78-00753.

**T-AM-Po100** TIME RESOLVED X-RAY DIFFRACTION STUDIES ON PURPLE MEMBRANE FROM HALOBACTERIUM HALOBIUM*
R. D. Frankel and J. M. Forsyth, Intr. by Robert S. Knox, Laboratory for Laser Energetics, University of Rochester, Rochester, NY 14623

Single shot nanosecond exposure x-ray diffraction patterns have been obtained on unstimulated and light stimulated stacked isolated Halobacterium halobium purple membrane (PM) fragments using a laser plasma x-ray source. The x-ray wavelength was 4.45Å and the samples were 60 μm thick. Patterns were recorded on an intensifier-aided two dimensional T.V. system. Both dried and hydrated PM stacks have been successfully used. Stimulation was provided by a train of frequency doubled (530 μm) green light pulses from a $Nd^{3+}$:YAG laser (rather than a single pulse) in order to achieve the maximum population of excited molecules. The delay between the stimulus and the x-ray flash varied between 1.0 - 50.0 milliseconds. Reversible changes have been observed between the diffraction patterns of unstimulated and stimulated specimens.

*Work supported, in part, by the National Institute of Health, the National Science Foundation and the Laboratory for Laser Energetics.

**T-AM-Po101** BILIPROTEINS: SOME RELATIONSHIPS AMONG AGGREGATION STATES, SPECTRA, AND EXCITATION-ENERGY TRANSFER. Robert MacColl and Donald S. Berns, New York State Department of Health, Division of Laboratories and Research, Albany, New York, USA

The key aggregation steps for C-phycocyanin consist of denatured subunits (α and β polypeptide chains), monomers, trimers, hexamers and stacks. For allophycocyanin the steps are denatured subunits, monomers and trimers. Ultimately these assembly states lead to the formation of phycobilisomes in blue-green and red algae. The spectroscopic changes that accompany each stage in the assembly can be monitored as a function of the ratio of visible to near-UV absorption. For C-phycocyanin, monomers and hexamers are the important levels in the modulation of tetrapyrrole conformation by protein-chromophore interaction. For allophycocyanin the monomers also have a less cyclic tetrapyrrole conformation than the denatured subunits, but the trimer spectrum may in addition include an important chromophore-chromophore interaction contribution. The effect of protein aggregation on the protein-chromophore interactions is the key to solar-energy harvesting and excitation-energy migration in photosynthesis.

T-AM-Po102 FACTOR ANALYSIS OF CHLOROPHYLL FLUORESCENCE IN PHOTOSYNTHETIC SYSTEMS
M.A. Marchiarullo and R.T. Ross. Department of Biochemistry, Ohio State University, Columbus, Ohio 43210

Factor analysis of fluoroscence spectra is an extension of the matrix analysis method of Weber (Nature (1961) 190,27). Unlike Weber's method, factor analysis permits determination of the component spectra. Factor analysis was performed on the room temperature fluorescence emission of whole algae (Chlorella and Scenedesmus), broken algae, chloroplasts isolated from market spinach, and photosystem I particles solubilized with Triton X-100. Parameters used as independent variables included excitation wavelength, emission wavelength, temperature, and the presence of different oxidizing and reducing agents. After the number of distinguishable species had been determined, the corresponding excitation and emission spectra were obtained by using a modified target transformation method to find appropriate linear combinations of the eigenvectors from abstract factor analysis. Analysis for the algae and spinach chloroplasts revealed three different fluorescing species. Spinach chloroplasts exhibited a component with a peak at 688 nm with a very prominent shoulder from 700-740 nm, similar to PSI-110 particles isolated by Mullet et. al. (Plant Physiol. (1980) 65, 814-822). Algae, either whole or disrupted by French press treatment, lacked the long wavelength shoulder.

**T-AM-Po103** SUPPRESSION VS COUNTERFLUX RECIPROCITY IN THE THERMODYNAMICS OF ACTIVE ION TRANSPORT.
K. M. Chapman, Neurosciences Sect., Div. Biology & Medicine, Brown University, Providence RI 02912

The basic problem in active Na-K transport is that the electrochemical potentials ($V-E_{Na}$) and ($V-E_K$) that drive diffusion cannot also utilize their free energy directly to get these ions back. The linear nonequilibrium thermodynamic theory of Kedem (1961) and others, further developed here, assumes the DeDonder affinity A for ATP hydrolysis is cross-coupled to drive active Na-K transport, resulting formally in V-independent active ion currents $g_{Nar}A$ and $g_{Kr}A$ accompanied by reciprocal ATP synthesis at V-dependent rates $-g_{Nar}(V-E_{Na})$ and $-g_{Kr}(V-E_K)$. From this I conclude: 1) the pump mediates a reciprocity arrangement whereby the electrochemical potentials make ATP at precisely the rate needed to power the transport, and affinity A drives the ions. 2) In the resting membrane the net ATP turnover is not the ATP used to fuel the pump, but is rather the metabolic cost of second law overhead. 3) The second law seems to require passive pump leaks for Na and K, and while these could act to suppress affinity-driven Na-K transport, experimental literature indicates instead the affinity-driven and passive ion fluxes are distinct, unidirectional counterfluxes. 4) If reciprocal ATP synthesis is likewise a counterflux back reaction, then forward ATP hydrolysis at the affinity-driven rate $g_rA$ (but not net turnover) may be considered stoichiometrically coupled to active ion transport. 5) If however reciprocity suppresses forward hydrolysis, then the pump is inherently nonstoichiometric in this sense and must transfer power from the electrochemical potentials without splitting ATP to free ADP and $P_i$. 6) If forward and back ATP reactions can be measured explicitly, the suppression vs counterflux question can be resolved. 7) In either case, ATP plays the role of a catalyst in the power transfer. Supported by USPHS Grant RR05664 and the Grass Foundation.

**T-AM-Po104** KINETICS OF CALCIUM EFFLUX FROM ISOLATED NERVE TERMINALS.
S. Sanchez-Armass* and M.P. Blaustein. Dept. of Physiol. Univ of Maryland, Med. Sch. Baltimore MD 21201 (Intr. by W.J. Lederer)

The properties of the Ca extrusion mechanism in presynaptic nerve terminals have been studied by measuring the efflux of Ca from rat brain synaptosomes loaded with $^{45}Ca$ during a brief depolarization (10 sec in 75 mM K, 20 $\mu$M $Ca^{2+}$ medium). The loaded terminals were diluted 16-fold into 0.1 mM EGTA media with varying concentrations of Na and Ca, and incubated for 2-120 sec at 20 or 30°C to determine $^{45}Ca$ efflux. Efflux was stopped by filtration and the filters were washed twice with cold Ca-free,145 mM Na solution with 1 mM $LaCl_3$. The initial $^{45}Ca$ load was determined by diluting 16-fold with cold Na- and Ca-free solution with 0.1 mM EGTA, followed by immediate filtration and wash. The decrease of $^{45}Ca$ on the filters, relative to the initial load, was taken as a measure of Ca efflux. In the absence of $Na_o$ (substituted by choline, N-$CH_3$-glucamine or Li) the rate of Ca efflux was 0.2 %/sec. $Na_o$ activated the Ca efflux with a $K_m \cong 30$ mM. We observed 2 phases of $Na_o$-dependent Ca efflux, with half times of 12 and 35 sec, respectively; both were blocked by 1 mM La. With 145 mM $Na_o$ the initial rate of Ca efflux was 5 %/sec. Ca efflux into either Na- free or Na-containing media was increased by $Ca_o$. With a large Ca load (100-200 $\mu$M $Ca^{2+}$ in loading solution), 5 $\mu$M FCCP, a mitochondrial uncoupler, increased the initial rate of Ca efflux,probably by releasing Ca from intraterminal stores. We conclude that nearly all of the (net) Ca efflux is $Na_o$-dependent, and is probable mediated by a Na-Ca exchange mechanism. Assuming that the entering $^{45}Ca$ exchanges completely with intra-terminal Ca, the $^{45}Ca$ efflux rate of 5 %/sec corresponds to about 0.5 pmoles/mg protein per msec. At this rate, the extrusion system could keep up with Ca entry at a firing frequency of about 300 spikes/sec. Supported by NIH. S.S-A. is a Fogarty Fellow and IMSS (Mexico) Fellow.

**T-AM-Po105** EVIDENCE FOR REVERSAL OF ACID EXTRUSION IN BARNACLE GIANT MUSCLE FIBERS.
J.M. Russell[1], W.F. Boron[2] and M.S. Brodwick[1]. Univ. of Texas Medical Branch, Galveston, TX 77550[1] and Yale Univ., New Haven, CT 06510[2].

A small dialysis tube, a pH-sensing microelectrode and a membrane-voltage microelectrode were placed inside a barnacle muscle fiber which was then dialyzed to an internal pH ($pH_i$) of 6.7-6.9 (normal $pH_i$ ~ 7.3). Under normal conditions of $pH_o$ 7.8 and $[Na]_o$ 465 mM, addition of external $HCO_3$ at constant $pH_o$ causes $pH_i$ to rise due to forward operation of the $pH_i$-regulating system (i.e., acid extrusion). This acid extrusion is accompanied by the net influx of Na and efflux of Cl and is blocked by SITS. We now report two conditions that cause an apparent reversal of the transport system (i.e., acid uptake), leading to a decrease of $pH_i$. The first is an application of $HCO_3$ in an acidic (pH 6.4), but otherwise normal, external solution. This caused a pronounced fall in $pH_i$ which was abolished by SITS. As expected if the transporter were reversed, the acid uptake rate was increased four-fold by raising nominal $[Na]_i$ from 0 to 75 mM, but was reduced to zero when extracellular Cl was replaced. The second means for apparently reversing the transporter is removal of external Na, in an otherwise normal, $HCO_3$-containing solution. This also caused a pronounced fall in $pH_i$ against the electrochemical gradients of $H^+$, $OH^-$ or $HCO_3^-$. This fall in $pH_i$ was blocked by SITS but was enhanced by reducing external $HCO_3$ and $pH_o$. Thus, acid uptake has the properties expected of the $pH_i$-regulating system running backwards: a dependence upon internal Na and external Cl, inhibition by SITS and an ability to generate a non-equilibrium $pH_i$.

Supported by NS 11946.

**T-AM-Po106** Na FLUXES ASSOCIATED WITH INTERNAL pH REGULATION IN BARNACLE MUSCLE. J.M. Russell and M.S. Brodwick, Dept. of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston, Texas, 77550

Unidirectional Na fluxes and internal pH were simultaneously measured in internally dialyzed barnacle giant muscle fibers. Ouabain (3 x $10^{-5}$M) was present throughout to inhibit Na fluxes mediated by the Na/K pump. The fibers were dialyzed to a relatively acidic value (~6.9). This had little effect on the Na influx until 6 mM $HCO_3$ (0.4% $CO_2$; pH 7.8) was added to the external fluid. This addition stimulated Na influx by 55±3 pmoles/$cm^2$. sec (p/cs) and caused the internal pH to rise about 0.15 units. Addition of external $HCO_3$ when internal pH was normal (7.3) had no effect on Na influx. The $HCO_3$-stimulated Na influx could be blocked by SITS and it required the presence of intracellular Cl. Sodium efflux was also stimulated by external $HCO_3$ when internal pH was acidic but not when internal pH was normal. The $HCO_3$-stimulated efflux averaged 12±2 p/cs. The $HCO_3$-stimulated efflux was also blocked by SITS, and it required the presence of external Cl. Furthermore, Na efflux could be stimulated when $HCO_3$ was presented intracellularly, a treatment which has little effect on internal pH. It has been hypothesized that internal pH regulation is accomplished by a system that causes a net influx of external Na and $HCO_3$ in exchange for internal Cl. The present results suggest that the system which mediates the afore-mentioned exchange is also capable of mediating fluxes in the opposite direction, i.e., Na and $HCO_3$ out of the cell in exchange for external Cl. Under conditions where this is the net effect, it would result in an acidification of the intracellular fluid. Supported by NS 11946.

**T-AM-Po107** CHLORIDE TRANSPORT MECHANISMS IN THE GUINEA PIG COCHLEA. H. Mori, T. Konishi. (Intr. by C.F. Chignell) Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709

Biophysical mechanisms involved in $Cl^-$ movement between the endolymph and perilymph have not been fully understood in the mammalian cochlea. In order to alter the electrochemical gradient for $Cl^-$ across the endolymph-perilymph barrier of the guinea pig cochlea, the perilymphatic space was perfused with $Na^+$-, $K^+$- or $Cl^-$-deficient artificial perilymph. The $Cl^-$ activity of the endolymph and endocochlear potential were simultaneously measured with a double-barreled $Cl^-$ sensitive liquid membrane electrode. The $Cl^-$ activity in the perilymph was also monitored with Ag-AgCl electrode. Small samples of the cochlear fluids were collected and the total chloride concentrations were determined by electrometric titration. The pre-perfusion $Cl^-$ activity of the endolymph and perilymph (the scala tympani and the scala vestibuli) were 97.8 ± 5.5 mEq/L, 92.0 ± 4.7 mEq/L and 93.9 ± 4.6 mEq/L respectively. The estimated $Cl^-$ activity coefficients of the endolymph and perilymph were approximately 0.74, suggesting that chloride in the cochlea fluid is not bound. When the electrochemical potential difference for $Cl^-$ between the endolymph and perilymph was modified with perilymphatic perfusions, no substantial changes in $Cl^-$ activity of the endolymph were observed. These results seem to suggest that leakage of $Cl^-$ is accompanied by longitudinal or radial water flow in the endolymphatic space, even though the possibility of a neutral or variable $Cl^-$ extrusion pump mechanism cannot be ruled out.

**T-AM-Po108** OSMOTIC EFFECTS ON INTRACELLULAR SODIUM AND CALCIUM ACTIVITY IN SHEEP CARDIAC MUSCLE M. Lado, S-S. Sheu, and H.A. Fozzard, Depts. of Medicine and the Pharmacological and Physiological Sciences, University of Chicago, Chicago, Ill. 60637.

Heart muscle cells behave like osmometers within a certain range of tonicity change, when the response is measured by change in intracellular potassium ion activity (Fozzard and Lee, J. Physiol 256:663-689,1976). We have measured steady-state intracellular sodium ion activity ($a^i_{Na}$) and intracellular calcium ion activity ($a^i_{Ca}$) in sheep ventricular muscle and Purkinje fibers during exposure to solutions of various tonicity (made by adjusting the sucrose concentration) by means of neutral liquid ion exchanger microelectrodes (ETH 227 for sodium, ETH 1001 for calcium). These results indicate that $a^i_{Na}$ changed less and $a^i_{Ca}$ changed more than predicted by the tonicity change. The $a^i_{Na}$ may initially respond to the volume change, but it is then adjusted by the Na pump to a new level that depends on the Na leak. Similarly, $a^i_{Ca}$ in the steady-state is determined by the sodium electrochemical gradient (Na-Ca exchange).

| | $a^i_{Na}$ (mM) | | $a^i_{Ca}$ (nM) | |
|---|---|---|---|---|
| Tonicity | Purkinje | Muscle | Purkinje | Muscle |
| 100% | 6.9±1.6(26) | 5.6±1.3(8) | 101±53(22) | 70±20(3) |
| 75% | 5.6 (81%) | 4.3 (71%) | 67 (67%) | - |
| 150% | 9.5 (138%) | 7.1 (127%) | 193 (191%) | 236 (337%) |
| 200% | 10.8 (151%) | 8.1 (145%) | 412 (408%) | - |

100% values are reported with S.E. and number of experiments in parens.

**T-AM-Po109** MEASUREMENT OF NET BIOGENIC AMINE UPTAKE INTO ISOLATED CHROMAFFIN GHOSTS BY TWO NOVEL METHODOLOGIES. R.G. Johnson, S. Hayflick, S.E. Carty, and A. Scarpa. Dept. Biochem-Biophysics, University of Pennsylvania, Philadelphia, PA 19104 USA.

Uptake of catecholamines and structurally related amines was measured by two newly-applied assay techniques; 1) the on-line kinetic measurement of amine uptake by a potentiometric electrode sensitive to catecholamines, and 2) filtration of the ghosts from the incubation medium and analysis of the effusate by HPLC. Chromaffin ghosts devoid of most of the endogenous intragranular matrix contents were formed from chromaffin granules isolated from bovine adrenal medulla. The addition of ATP to the ghosts suspended in a chloride containing medium at pH 7 resulted in the generation of a $\Delta$pH, inside acidic, of 1 pH unit, as measured by [$^{14}$C]methylamine. The addition of 10 $\mu$M epinephrine to the ghost suspension after the $\Delta$pH reached steady state values produced a rate-dependent accumulation as measured by both amine assay techniques. Collapse of the $\Delta$pH by ammonia addition was dependent upon the magnitude of the $\Delta$pH and could be inhibited by tyramine. Similar results were obtained for a wide variety of biogenic and pharmacologic amines, including dopamine, norepinephrine, isoproterenol, and metaraminol. These results demonstrate unequivocally that large magnitude net accumulation of biogenic amines into chromaffin ghosts does occur and suggests that the techniques outlined offer advantages over the widely utilized method of radiochemically labeled amine distribution measurements. Application of these techniques to the study of amine transport will permit further characterization of the properties of the amine transporter and the relationship of the electrochemical proton gradient to the equilibrium amine gradient (This work was supported by NIH grant CA-24010).

**T-AM-Po110** NMR STUDIES OF NUCLEOTIDE AND AMINE STORAGE IN DENSE GRANULES OF PIG PLATELETS. K. Ugurbil, M. Fukami, and H. Holmsen. Dept. of Biochemistry, Columbia University, NY, NY 10032 (KU) and Specialized Center for Thrombosis Research, Temple University, Philadelphia, PA 19140 (MF and HH).

Proton nuclear magnetic resonance (NMR) studies have been conducted on dense granules isolated and purified from pig platelets. NMR spectra obtained from $HCl O_4$ extracts of dense granules show that these vesicles sequester approximately 1.5 times as much histamine as serotonin. Resonances of the H2 and H8 protons of the adenine ring, H1' proton of the ribose moiety, and the aromatic protons of serotonin have been identified in spectra of intact granules. Their positions indicate that the nucleotide aromatic rings are highly stacked with each other and with serotonin. Association of serotonin with the nucleotides is also confirmed by the presence of an intermolecular nuclear Overhauser effect (NOE) between serotonin and nucleotide protons. Intramolecular NOEs between the H1', and the H2 and H8 protons of nucleotides show that the nucleotides are in the anti conformation. These results are consistent with the proposed existence of complexes with high apparent molecular weights involving the nucleotides and serotonin in the interior of the dense granules.
(Supported by NIH Grant No. H126089.)

**T-AM-Po111** ION-ION INTERACTIONS IN CONTINUUM STATISTICAL MECHANICAL FORMALISMS FOR MEMBRANE ION TRANSPORT THEORY. K. Cooper, E. Jakobsson, P. Wolnyes, Department of Physiology and Biophysics, Program in Bioengineering, and Department of Chemistry, University of Illinois, Urbana, Illinois 61801.

Ion-ion electrostatic interactions are accounted for in the Nernst-Planck electrodiffusion equation by including the charge of the permeant ions in the Poisson equation, which in turn contributes to the voltage gradient term in the Nernst-Planck equation. The steps in this derivation will be displayed. The expressions for the mutual dependence of voltage and concentration gradients (i.e., self-consistent field equations) are:

$$\frac{d^2c}{dx^2} + z\frac{dc}{dx}\frac{d\phi}{dx} + zc\frac{d^2\phi}{dx^2} = 0$$

$$\frac{d^2(\varepsilon\phi)}{dx^2} = \frac{(C + C')}{\varepsilon_o}$$

C - permeant ionic concentration
$\phi$ - normalized potential
C' - all other charge in system other than linear dielectric of permeant ion
$\varepsilon$ - dielectric coefficient

The range of validity of this theory will be discussed.

The Nernst-Planck equation does not include all velocity-dependent ion-ion interactions. Approaches to account for such interactions include addition of a time-independent velocity-dependent term (frictional term) and construction of a fluctuating force term. These two formal approaches will be displayed and their relationships to each other discussed.

**T-AM-Po112** THE ELECTROCHEMICAL PROTON GRADIENT IN ISOLATED DENSE GRANULES OF BOVINE ANTERIOR PITUITARY. S.E. Carty, R.G. Johnson, & A. Scarpa. Dept. Biochem-Biophys., University of Pennsylvania, Philadelphia, PA 19104 USA.

Intact anterior pituitary dense granules containing growth hormone and prolactin were isolated and purified from other subcellular organelles using a newly developed isotonic continuous density gradient of Percoll. As measured by radioisotopic distribution methods, both growth hormone and prolactin granules exhibited an internal pH of 5.10 when suspended in isotonic media at physiological pH, and exhibited a high internal buffering capacity. Varying the external pH produced a corresponding change in the transmembrane pH gradient ($\Delta$pH) which could be completely collapsed by the stepwise addition of 40 mM $(NH_4)_2SO_4$. In the presence of 2 mM MgATP a transmembrane potential ($\Delta\Psi$), inside positive, of 60-70 mV was generated and sustained for over 30 min., then collapsed by the addition of uncoupler. Unlike biogenic amine uptake into other subcellular storage granules, however, accumulation of radiolabeled catecholamines into pituitary dense granules was found to proceed only in response to the magnitude of the $\Delta$pH, and was unaffected by the presence of the $\Delta\Psi$. Moreover, accumulation of amines was not inhibitable by reserpine. These results suggest that the membrane of the dense granules is highly impermeable to protons and contains a proton translocating ATPase responsible for generation and maintenance of the $\Delta$pH and $\Delta\Psi$ gradients. The physiological function of the markedly large electrochemical proton gradient observed in these granules thus does not appear to function as a mechanism for accumulation of biogenic amines, but may have important implications for the synthesis, physiologic maintenanace, and accumulation of the intragranular matrix components of pituitary growth hormone and prolactin granules. (Support:NIH-CA-24010; HL-18708)

**T-AM-Po113** THE DEPENDENCE OF THE $Ca^{++}$ EFFLUX FROM SINGLE APLYSIA NEURONS ON EXTRACELLULAR Na. L. Satin, Department of Biology, UCLA, Los Angeles, Ca. 90024

Although Ca efflux has been extensively studied in the squid axon and in barnacle muscle (Baker and McNaughton (1976). J. Physiol. 259, 103-144; Russell and Blaustein (1974). J. Gen. Physiol. 63, 144-167) it has not been previously studied in single nerve cell bodies. $^{45}Ca$ was injected into Aplysia giant (100-800 $\mu$m diameter) neurons by passing small (10-30 nA) constant current pulses of 0.5 sec duration through an intracellular micropipette filled with 10mM carrier-free $^{45}CaCl_2$. Membrane potential was monitored conventionally with a second micropipette (filled with 3M KCl) and in some experiments, potential was held constant with a third electrode (filled with 3M KAc) which could pass DC polarizing current. The abdominal ganglion was dissected and secured in a chamber where saline could be superfused over the ganglion at a constant rate and could be collected at an outflow point every 5 minutes. Aliquots of saline were assayed for $^{45}Ca$ by using conventional liquid scintillation counting techniques. Experiments were conducted for 2-3 hours at 22°C, and cells were usually excitable at the end of the experiment. Ca efflux (expressed as cpm/5 min.) rose smoothly during the course of injection and then declined slowly following the injection. If $Na_o$ was completely replaced with an equi-osmotic amount of Tris, the efflux was reduced by 38 $\pm$ 9 per cent (mean $\pm$ s.d.; 8 cells). This reduction was not due to the hyperpolarization usually seen in Na free, since holding the potential constant with injected outward current did not prevent a reduction in the observed efflux. It is concluded that Ca efflux is partially coupled to $Na_o$ in Aplysia as it is in squid axon, at least over some range of $(Ca^{++})_i$ (Blaustein and Hodgkin (1969). J. Physiol. 200, 427-527; Baker and McNaughton (1976), J. Physiol. 259, 103-144).

**T-AM-Po114** PLATEAU MEMBRANE CURRENTS IN SINGLE HEART CELLS. I. Josephson, J. Sanchez-Chapula. Department of Physiology and Biophysics, UTMB, Galveston, TX 77550.

The ventricular action potential is brief in rat compared to most mammalian species (such as guinea pig); it is characterized by an early repolarization followed by a low plateau. It was, therefore, of interest to examine the membrane currents which contribute to this unusual ventricular plateau. Voltage clamp experiments were conducted on single ventricular myocytes which were isolated enzymatically from adult rat and guinea pig hearts. Membrane currents were obtained using a single-microelectrode (switching) voltage clamp circuit and were compared with a standard two-microelectrode voltage clamp to insure their validity. In rat heart cells, depolarizing voltage clamp steps above -30 mV elicited an early outward current (blocked by 4-AP) which overlapped in time with the slow inward current. The early outward current was voltage-inactivated at -50 to -40 mV, thereby permitting a clear examination of the slow inward current ($I_{si}$). The threshold for $I_{si}$ in both rat and guinea pig cells was -35 mV, and the peak current was maximal at 0 to +10 mV. $I_{si}$ decayed with two components; the second, maintained $I_{si}$ component was more pronounced in the guinea pig. Double pulse experiments were designed to study the effects of varying the amplitude and duration of a conditioning prepulse on a second, test pulse. The results from these experiments suggest that a significant amount of inactivation may reflect $Ca^{++}$ entry during $I_{si}$.

In summary, it appears that an early outward current contributes to the rapid repolarization phase of the rat ventricular action potential, whereas a slowly-inactivating, slow inward current maintains the guinea pig plateau at more positive potentials.

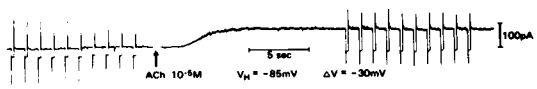
Supported by NIH grants NS 11453 and HL 25145.

**T-AM-Po115** EFFECTS OF LIDOCAINE AND BENZOCAINE ON CARDIAC SODIUM CURRENT. J. Sanchez-Chapula, Y. Tsuda, I. Josephson and A.M. Brown. University of Texas Medical Branch, Galveston, Texas 77550.

The effects of local anesthetics on sodium currents of enzymatically isolated, single rat ventricular cells were studied. A suction pipette was used for passing current and internal perfusion, and membrane potential was measured by microelectrode (10-15 M Ω tip resistance). This voltage clamp technique allows an accurate membrane potential measurement and avoids the problem of access resistance which may arise when using only suction pipette. The external sodium concentration was reduced to 40 mM and the temperature to 23°C to further insure good voltage control. Potassium currents were blocked by applying $CsCl_2$ (100 mM) externally and Cs-Aspartate (140 mM) internally. Two local anesthetics (lidocaine and benzocaine) were compared in experiments designed to test recovery from inactivation and use-dependent block of $I_{Na}$. The recovery from inactivation in presence of lidocaine (20 μ M) had a fast phase and a slow phase. The latter was markedly slowed compared to control conditions, it followed a time course which could be fitted by an exponential. The tau was ~400 msec following a 50 msec conditioning pulse, and ~950 msec following a 500 msec conditioning pulse. Lidocaine produced a tonic block of ~30%; applying 50 msec pulses at a frequency of 2 Hz, it produced an additional use-dependent block of ~10%. When the duration of the pulses was increased to 500 msec at a frequency of 1 Hz, the additional use-dependent effect produced by lidocaine was ~25%. On the other hand, benzocaine (100 μ M) produced only a small lengthening of the recovery time constant. Benzocaine (100 μ M) produced a tonic block of ~40% using pulses of 500 msec duration at a frequency of 1 Hz, however, it produced only a small additional use-dependent effect of ~10%. Supported by NIH grants NS 11453 and HL 25145.

**T-AM-Po116** ACETYLCHOLINE-INDUCED OUTWARD CURRENT IN SINGLE BULLFROG ATRIAL CELLS. Y. Momose, G. Szabo and W. Giles (Introduced by F. Wong). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

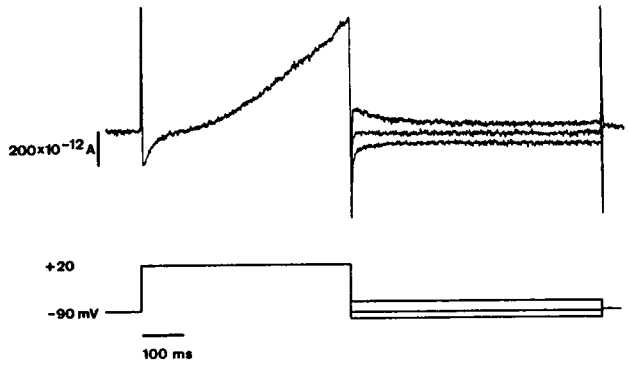
Enzymatically dispersed single bullfrog atrial cells (Hume and Giles, J. Gen. Physiol. 78: 19-42, 1981) have been voltage-clamped using a one-microelectrode technique, and biophysical properties of a steady outward current produced by activation of muscarinic receptors have been investigated. The muscarinic current has the following properties: (1) a dose-response curve for acetylcholine extending from $5x10^{-8}$M to $1x3^{-3}$M, with a a $K_M$ near $3x10^{-6}$M, (2) complete inhibition by atropine ($10^{-7}$M), and partial inhibition by $Ba^{++}$ ($10^{-5}$ to $10^{-4}$M), (3) activation by muscarine (>$10^{-8}$M) and bethanecol (>$10^{-7}$M), but not by hexamethonium, (4) a reversal potential between -95 and -105 mV in 2.5 mM $[K^+]_o$.




ACh-induced current records (see Figure) are more 'noisy' than controls; and a pronounced relaxation is recorded when hyperpolarizing clamp steps are terminated. Preliminary analysis of kinetic and noise data imply the presence of ACh-activated channels with a conductance of about 2 pS and mean open time of 80 msec. Single channel recordings in patch clamp experiments appear to be consistent with these data. However, both relaxation and noise experiments also reveal more rapid (~ 5 msec) electrical events, presumably corresponding to fluctuations of 'open' ACh-activated channels. (Supported by NIH HL-27454 and HL-24820).

**T-AM-Po117** OUTWARD CURRENT IN SINGLE BULLFROG ATRIAL CELLS. J. R. Hume and W. Giles, University of Texas Medical Branch, Galveston, Texas 77550.

An enzymatic dispersion technique yields quiescent single atrial cells having normal resting potentials and action potentials; and a dc space constant of approximately 900 μ(Hume and Giles, J. gen. Physiol., 78: 19-42). A single microelectrode voltage clamp technique similar to that of (Hamill et al, Pflügers Archiv. 391: 85-100.) was utilized to study the time- and voltage-dependence of the outward current.




Graded, 1 sec., voltage steps were applied from a holding potential of -80 mV. The resulting tail currents yield an isochronal activation curve extending from -30 to +30 mV. Individual 'tails' decay with a single exponential time course ($\tau$ = 450 msec., at -60 mV). The outward current turns on with considerable 'lag' in the plateau range of potentials (-10 to +20 mV).

Double pulse protocols were used to estimate $E_{rev}$; values of -90 to -95 in 2.5 mM $[K^+]_o$; suggest that potassium is the major charge carrier of the outward current in atrium. (Supported by NIH HL 27454 and AHA 81-835).

**T-AM-Po118** EFFECTS OF COPPER IONS ON CARDIAC ACTION POTENTIALS IN FROG ATRIAL FIBERS. K.-S. Tan and C.E. Challice, Departments of Pharmacology and Physics, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

The effects of copper ions on cardiac electrical activity in frog atrial fibers were studied at room temperature using the double sucrose gap technique. In the presence of $Cu^{++}$ ions (> 10μM), the action potential peak amplitude was depressed, this depression being both concentration and time dependent. Low concentrations of $Cu^{++}$ ions (< 50μM) produced a biphasic effect on the action potential duration. An initial increase was observed, followed by a depression. Above 50μM of $Cu^{++}$ ions, a single depressant phase on action potential duration was observed; the higher the concentration, the greater the depression. The membrane resting potential was insignificantly changed at low concentrations of $Cu^{++}$ ions, but above 60μM $Cu^{++}$ ions, the resting potential was depolarized by 7 ± 2 mV and this depolarization further increased when $\{Cu^{++}\}_0$ was increased. Washout did not produce recovery; instead a further depression of both action potential peak amplitude and duration were observed, even following an extended period of washout (> 1 hr.).

These results suggest that copper ions may compete with $Ca^{++}$ ions during the transmembrane movement of ions within the action potential cycle, affecting the plateau of the action potential. They further suggest that the copper ions may induce irreversible changes in the cardiac membrane, inhibiting its normal functioning.

Supported by the Natural Sciences and Engineering Research Council of Canada and Alberta Heritage Foundation for Medical Research.

**T-AM-Po119** EXTRACELLULAR CALCIUM ION DEPLETION IN FROG VENTRICLE. K.Dresdner,R.P.Kline,and J. Kupersmith. Depts.of Pharmacology and Medicine,Mount Sinai Med.Sch.,CUNY,NY,NY,10029

Using double barrel calcium ion ($Ca^{++}$)selective electrodes (Ca-ISEs),we recorded extracellular calcium concentration ($Ca_0$) and electrical potential in frog (Rana pipiens) ventricular strips (~ 1-2mm dia.)perfused with Ringers solutions (0.2 or 0.05mM $Ca^{++}$) and stimulated at 1.2 to 60 beats/minute. Ca-ISEs (tip o.d.=2-3 microns) were constructed from theta capillaries.Selective barrels were silanized with 2.3% DC200 in xylene(v/v),filled at tip with Simon's neutral carrier resin,and backfilled with 0.1M $CaCl_2$ or Ringers. Reference barrel was backfilled with 0.1M NaCl. Selective barrel time constants were 100-400 msec (with capacitive compensation), measured by applying square pulses from capacitively coupled ramp generator. Ca-ISEs were Nernstian in range pCa 3-5 with constant interfering ions (118$Na^+$,3$K^+$,2$HCO_3^-$,121$Cl^-$,in mM).Maximum error from changes in interfering ions during beating was estimated as 4% by varying $Na^+$(118 to 101mM) and $K^+$(3 to 20 mM)in calibrating solutions. During single isolated beats (1.2/min),$Ca_0$ depletions were as large as 45 micromolar (0.20 to 0.155mM), but were reduced for faster steady state stimulus rates or lowered bath $Ca^{++}$. For tip locations with no single beat $Ca_0$ fluctuations,slow depletions were seen as rate was increased from control(12/min)to 24-60/min.Rate dependent depletions were maximal in 3-8 minutes, slowly returned to bath levels with continued beating (20-30 minutes), and often overshot bath levels on return to control rate. Maximum depletions for highest rates and deep tip positions were 130-180 micromolar (0.2 to 0.070-0.020mM), and were reduced by lowering bath $Ca^{++}$. During 30 minute exposure to contracture fluid (100$K^+$,21$Na^+$,0.2$Ca^{++}$ 2$HCO_3^-$,121$Cl^-$,in mM) $Ca_0$ depleted with a magnitude comparable to the highest stimulation rates.

**T-AM-Po120** EXTRACELLULAR POTASSIUM SELECTIVE ELECTRODE MEASUREMENTS IN VOLTAGE CLAMPED SMALL CANINE PURKINJE FIBERS: R.P. Kline, I. Cohen, R. Falk, and J. Kupersmith, Mount Sinai Med. Ctr., CUNY, N.Y., N.Y. 10029 and SUNY Med. School, Stony Brook, L.I., N.Y. 11794

Membrane current (I) and transmembrane potential (Vm) were measured in small canine Purkinje fibers (radius<0.2mm) with the two microelectrode voltage clamp while simultaneously monitoring extracellular potassium activity (Ko) and extracellular potential (Vo) at the tip of a double barrel potassium selective microelectrode (K-ISE). The ratio of the change in Vo to the change in Vm for voltage clamp steps between any two voltages was used as an indication of the relative extracellular resitance for various K-ISE tip locations. For small values of the ratio ($\Delta Vo/\Delta Vm$ =0.01 to 0.05), monotonic slow changes in Ko were seen with comparable time course to the slow time dependent currents and the cylindrical diffusion time constants of the cylindrical preparations (approximately 1-4 seconds). Total Ko accumulations (for 20-40 millivolt depolarizing clamp steps to the plateau potential range) or depletions (for negative potential clamp steps to the diastolic potentials) were about 0.5 mM for 5 second clamp steps. For clamp steps to the plateau for a given negative holding potential, steps which produced larger currents also resulted in more accumulation. Similarly, for hyperpolarizing steps to diastolic potentials from a given holding potential, more negative clamp steps generated larger depletions. For K-ISE tip locations facing larger extracellular resistances (as indicated by $\Delta Vo/\Delta Vm$ values =0.05-0.25), the slow Ko accumulations were now superimposed on an initial larger and more rapid Ko fluctuation. Ko changes up to 5 mM were seen for above protocols with time constants for this fast phase being approximately 100-200 milliseconds.

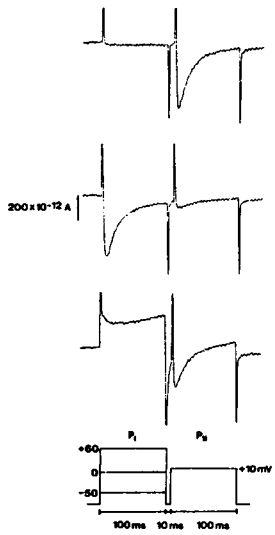
**T-AM-Po121** SLOW GATING OF THE PACEMAKER CURRENT IN CANINE PURKINJE FIBERS. N.Mulrine, I.Cohen, & R.Falk (Intr. by P.G.Lefevre), Dept. of Physiology & Biophysics, SUNY at Stony Brook,New York 11794

Analysis of pacemaker currents in cardiac Purkinje fibers has relied on the approach used to analyze squid permeability (Hodgkin and Huxley,1952,J.Physiol. 117,500-544). The channel which carries the pacemaker current is assumed to exist in only 2 states- open or closed. Transitions between the open and closed states occur via a first-order gating reaction with a time constant that is determined solely by the membrane potential, and varies between several hundred msec and a couple of seconds in the diastolic range of potentials. In the present study, we demonstrate that a slow process occurring over one or more minutes modulates by up to a factor of two the amplitude of the time-dependent current observed during pulses to diastolic potentials.

We used the 2 microelectrode voltage clamp technique to study currents in the pacemaker range of potentials in canine cardiac Purkinje fibers. We recorded the time-dependent current elicited during hyperpolarizing test pulses of 5-second duration, applied during cycles of 30-90 seconds in length. We initiated the pulsing cycle by either shifting from a relatively depolarized holding potential to a more negative holding potential, or applying a 5-second depolarizing test pulse. The amplitude of time dependent current decreased progressively with successive hyperpolarizing pulses, reaching a steady value only after 2-6 test pulses. In normal Tyrode, extracellular $[K^+]$ fluctuations can distort the pacemaker current. Addition of barium to the bathing Tyrode blocks background $K^+$ permeability and may reduce the distortion of the pacemaker current due to $[K^+]$ fluctuations. We obtained similar evidence for slow "gating" of the pacemaker current in barium containing Tyrode.

**T-AM-Po122** TURN-OFF OF A TTX-RESISTANT INWARD CURRENT '$i_{Ca}$' IN SINGLE BULLFROG ATRIAL CELLS. J.R. Hume and W. Giles, (Introd. by S.L. Hamilton), Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Tx 77550.

Our previous two-microelectrode voltage-clamp measurements of '$i_{Ca}$++' in enzymatically dispersed single bullfrog atrial cells (J. Physiol. 317: 53P, 1981) have shown that: (i) it is strongly modulated by increases or decreases in $[Ca^{++}]_o$ (ii) it is significantly inhibited by $Mn^{++}$, $Cd^{++}$ and D-600 (iii) its magnitude at 0mV ($250\text{-}300 \times 10^{-12}$A) is sufficient to generate the observed $dV/dt_{max}$ of the 'slow response'. Recent voltage-clamp measurements of $i_{Ca}$++ turn-off in isolated mammalian ventricular cells have been interpreted in terms of a Hodgkin-Huxley inactivation mechanism (Isenberg and Klöckner, Nature, 284: 358-360, 1980). We have studied the turn-off of $i_{Ca}$++ in single atrial cells and find consistent departures from the predictions of a conventional 'inactivation' scheme. (i) When $[Ca^{++}]_o$ is increased 3x (to 7.5mM) the rate of turn-off is accelerated about 50% (ii) Double pulse experiments (see Figure) show that as $P_1$ is made very positive (>+60mV), the current elicited by $P_{II}$ increases. These data indicate that the turn-off of $i_{Ca}$ is at least partially controlled by $Ca^{++}$ entry, a phenomena originally demonstrated in Paramecium and in Aplysia neurones. (Supported by NIH HL 27454).



T-AM-Po123

Abstract rescheduled to T-AM-Po115

**T-AM-Po124** COMPARISON OF NORMAL AND FAILING CARDIAC MUSCLES: THE DRIVE RELATED CHANGES IN EXTRACELLULAR POTASSIUM ACTIVITY. Francis G. Martin, Steven R. Houser, Thomas A. Marino, and Alan R. Freeman, Departments of Physiology and Anatomy, Temple University School of Medicine, Philadelphia, Pennsylvania, 19140.

It has been shown that isolated hearts subjected to pressure overload lose potassium and that the cells of hearts with pressure overload induced hypertrophy and failure have depolarized resting potentials. In order to study these phenomena at the cellular level we have measured the drive related changes in the extracellular potassium activity in both normal and failing cat papillary muscles and trabecullae using ion selective microelectrodes and morphometric techniques. Our findings indicate that muscles from both types of animals begin building up extracellular potassium immediately upon the onset of drive. Eventually, the potassium activity reaches a maximum and then returns toward a lower steady state level. This pattern of extracellular potassium accumulation takes significantly longer to unfold in failing muscles than it does in the normal muscles and the final steady state level of accumulation is significantly higher in the extracellular spaces of failing tissue than in those of normal tissue. However, the maximum extracellular potassium activity reached in failing muscles tends to be lower than the maximum reached in normal muscles. These differences in the patterns of drive related extracellular potassium accumulation between the two cardiac states are consistent with previously reported differences in the patterns of drive related changes in the resting membrane potentials measured in normal and failing cardiac muscles. They can be explained by a measured change in the volume of the extracellular compartment and an increased ionic load on the Na/K pump. (Supported by NIH #HL22673)

**T-AM-Po125** PRESERVATION OF ELECTROPHYSIOLOGIC CHARACTERISTICS OF THE FETAL DOG HEART IN CULTURE Richard B. Robinson and Peter Danilo, Jr., Dept. of Pharmacology, Columbia Univ., NY

Although cell cultures are well suited for studies of the control and modulation of membrane characteristics, comparisons between cell culture and the intact heart are hindered by the limited species from which cardiac cells have been cultured. We have developed monolayer cultures from mid-term fetal dog ventricle and find that the electrophysiological characteristics of the source tissue are preserved. The intact ventricle paced at a cycle length (CL) of 0.5 S and the spontaneously active culture with mean CL=1.8±0.1 S ($\bar{x}$±S.E.) have maximum diastolic potentials of -71±2 mV and -70±1 mV, respectively. Likewise, action potential amplitude is 88±1 and 98±2 mV in the two preparations. Although the intact tissue shows a higher rate of rise of phase 0 ($\dot{V}$max), 111±11 and 77±5 V/S respectively, $\dot{V}$max of cultured cells is sensitive to tetrodotoxin (TTX), a characteristic typical of intact cardiac tissue but which is often lacking in monolayer cultures. TTX, 1 mg/L, reduces $\dot{V}$max by 77±3% without significantly altering other action potential parameters, but spontaneous CL increases 108%. Finally, action potential duration at ½ amplitude (APD50) is 144±2 mS in the intact ventricle and 250±11 mS in culture. However, the cultures show a marked dependence of APD50 on spontaneous CL (r=0.81), such that for a CL of 0.5 S calculated to match the pacing rate of the intact preparation, APD50 of the cultures would be 165 mS. This is in marked contrast to what is seen in the more common rat heart cultures, where APD50 is less than 100 mS at comparable CL, suggesting that unique species characteristics are preserved in culture. We conclude that canine cardiac cultures are a useful preparation for studying the in vitro development of membrane electrical properties. (Supported by HL-12738, HD-13063 and the New York Heart Association)

**T-AM-Po126** $Ca^{2+}$-TRANSPORT AND THE GENERATION OF TENSION IN $Na^+$-DEPLETED FROG VENTRICULAR STRIPS. T. Yanasisawa & M. Morad, Dept. of Physiol., Univ. of Penna., Phila., PA 19104

Frog ventricular strips were bathed in $Na^+$-free solution (Tris-Cl or LiCl) for 1-2 hrs. $(Na^+)i$ was measured to be about 20-50μM. KCl depolarization or clamp-induced depolarization produced large tensions which were maintained for 2-5 seconds. In $Na^+$-depleted strips tension-voltage relation changed from a monotonically increasing relation, observed in $Na^+$-rich solution, to a bell-shaped curve. Tension peaked around 0 mV and decreased back to baseline tension around +70 mV. The voltage-dependence of tension in $Na^+$-depleted strips was similar to that measured for the slow TTX-insensitive inward current (Isi). The effect of pharmacological interventions on the tension-voltage relation and the I-V relation of Isi were similar. Adrenaline (10μM), caffeine (5mM), theophylline (1.5mM), and elevated $(Ca^{2+})_o$, all increased tension and Isi. Acetylcholine (1μM), divalent cations ($Cd^{2+}$,0.3mM; $Ni^{2+}$,2mM; $Co^{2+}$, 2mM; $Mn^{2+}$,20mM) and organic Ca-antagonists (D-600, 10μM & Diltiazem, 10μM) reduced both tension and Isi. These results suggest that the tension in $Na^+$-depleted strips is correlated with the activation of Isi. There is, however, discrepancy between the time course of Isi (400ms) and time course of tension (2-5sec), even in $Na^+$-depleted strips. This result indicates that Ca-channel may inactivate more slowly and thus transport sufficient $Ca^{2+}$ to activate tension. The rate of relaxation in $Na^+$-depleted strips was about 30 times slower than in $Na^+$-rich preparations. Although adrenaline had a strong relaxant effect in normal strips it failed to enhance the rate of relaxation in $Na^+$-depleted preparations. If it is assumed that in $Na^+$-depleted strips the SR activity is not impaired, then the results suggest that the $Na^+$-gradient is required for both removal of $Ca^{2+}$ and the relaxant effect of adrenaline. In this respect adrenaline may enhance relaxation by modifying the $Na^+$-gradient through stimulation of the $Na^+$ pump.

**T-AM-Po127** EFFECTS OF INTRACELLULAR $Ca^{2+}$ RELEASE ON MEMBRANE CURRENTS IN ATRIAL FIBERS. M. Morad & J. Maylie, Dept. of Physiology, Univ. of Penna., Phila., PA 19104

It is generally accepted that $Ca^{2+}$ release from the SR contributes to development of tension in mammalian heart. We set out to test whether contraction-related Ca-release from the SR would affect surface membrane currents. Thin trabeculae (200-300μm) from Elephant-Seal atria were placed in a single Sucrose-gap voltage-clamp apparatus. A large transient outward current ($I_{to}$) was found to activate at potentials positive to -30mV, reached maximum at +30mV and decayed back to zero at +65mV. $I_{to}$ ranged in magnitude from 0.4-0.8μA/cm$^2$, and lasted for 200-400msec. The voltage-dependence of $I_{to}$ is similar to that of tension. $I_{to}$ followed the development of $I_{si}$, but preceded the generation of tension. Positive to +10mV $I_{to}$ showed beat-dependence similar to tension. $I_{to}$ was strongly enhanced by epinephrine, increased with increasing $(Ca^{2+})_o$ and was suppressed by tetracaine, $Mn^{2+}$ and caffeine. 1mM caffeine suppressed the beat-dependent kinetics of both $I_{to}$ and tension, without altering their voltage-dependence. 5mM caffeine blocked $I_{to}$, reduced the rate of rise of tension and slowed the rate of relaxation and abolished post-clamp potentiation. The suppression of $I_{to}$ by caffeine paralleled the effect of the drug on suppression of the 2nd component of the birefringence signal. Since the tension-voltage relation in this tissue is also bell-shaped, the results suggest that $I_{si}$ releases intracellular $Ca^{2+}$ stores, generating tension and $I_{to}$. Agents which increase the size of these stores enhance $I_{to}$ and those which block their release suppress $I_{to}$. Suppression of $I_{to}$ and the 2nd component of the birefringence signal by caffeine suggests that potentiation of tension by caffeine is mediated by a suppression of Ca-uptake rather than enhancement of SR Ca-release. Although $I_{to}$ may result from a Ca-induced increase in $g_k$, we have no direct experimental evidence for it.

**T-AM-Po128** KINETIC MEASUREMENTS OF A MAINTAINED INWARD CURRENT IN FROG VENTRICLE. L. Tung and M. Morad, Dept. of Physiology, Univ. of Penna., Phila., Pa 19104

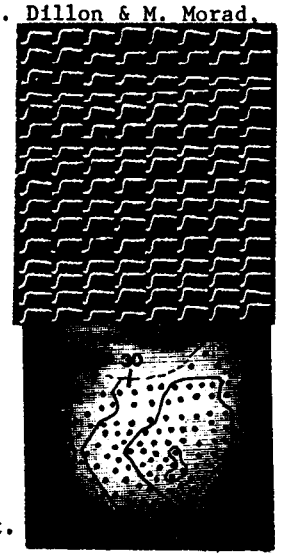
The slow inward current (Isi) in frog ventricle was studied using the single sucrose gap technique. It is generally assumed that Isi represents a channel which transiently activates and then inactivates. Since the amplitude of Isi increases with $(Ca^{2+})_o$ and decreases with $Ca^{2+}$-blockers, Isi is thought to be a transient calcium current. We set out to measure the kinetics of Isi and found several inconsistencies in attributing all of the TTX-insensitive inward current ("Isi") to a $Ca^{2+}$ channel with activation and inactivation gates. The following observations suggest in part the presence of a maintained inward current: 1) Total membrane conductance increases during the time course of "Isi", suggesting that the time-dependent behavior of "Isi" is due not to an inactivation process but rather to the activation of an outward-going current (Tung & Morad, Biophys. J. 1981). 2) The envelope of "tail" currents (clamping back to rest) decays with a time constant on the order of seconds, suggesting an inward channel which lacks an inactivation gate. This maintained inward current is not due to the overlap of activation and inactivation curves, since the magnitude of the "tail" current does not decrease, even after 400ms, during clamps to increasingly positive potentials. In preparations in which $Ca^{2+}$ was replaced by $Sr^{2+}$ or $Ba^{2+}$, the outward current was suppressed, while the envelope of "tail" currents retained its maintained behavior. 3) The "inactivation" curve for "Isi" is bell-shaped, rather than S-shaped. According to this new interpretation, the maintained inward current activates with a time constant of about 20ms at 0 mV and deactivates with a time constant of about 50ms at rest. The slow outward current component activates with a time constant of about 150ms at 0 mV and deactivates with a time constant of about 100ms at rest. The results may also be consistent with a coupled transporter.

**T-AM-Po129** TRANSIENT DEPOLARIZATION OF ATRIAL FIBERS FOLLOWING EXPOSURE TO ACETYLCHOLINE.
H. Nishiye*, P.F. Cranefield and D.C. Gadsby, Rockefeller University, New York, NY 10021.

Quiescent cells of canine atrial trabeculae or coronary sinus strips (4mM $K_o$; resting potential -50 to -70 mV) hyperpolarize during exposure to ≥10nM acetylcholine (ACh); on sudden removal of ACh, that hyperpolarization declines and is followed by a small (1-15 mV) depolarization which reaches a peak about 30s after ACh removal and then decays slowly with an approximately exponential time course of average half-time 78s (SD±27s, n=44). The peak amplitude of this post-ACh depolarization increases and appears to saturate, as the preceding exposure to ACh is prolonged from 5s to 200s, with little change in the time course of decay. Peak amplitude of the post-ACh depolarization increases with ACh concentrations which also increase the ACh-induced hyperpolarization. When the ACh-induced hyperpolarization is diminished by 1μM atropine, the post-ACh depolarization is similarly attenuated; however, preliminary experiments in which the membrane potential was held constant in very small coronary sinus strips by use of a two-microelectrode voltage clamp indicate that the hyperpolarization is not necessary for subsequent appearance of the post-ACh depolarizing shift in net membrane current. The post-ACh depolarization is not abolished immediately after inhibiting the Na/K pump with 2μM acetylstrophanthidin or K-free superfusion, suggesting that it does not reflect pump-mediated restoration of Na or K gradients. The post-ACh depolarization appears to be unaffected by 10μM curare, 10μM phentolamine, or 10μM ACC 8052 (new β-blocker), but it is somewhat attenuated by 1μM propranolol. It is almost unchanged by replacing virtually all $Cl_o$ by isethionate and is not abolished by 4μM D600 or 10μM TTX nor by replacing $Ca_o$ (2.7mM) by Mn (2.7mM). It persists, albeit diminished, even in 4mM K,Na-free, sucrose-containing solution in which all Ca is replaced by Mn. Under appropriate conditions, the post-ACh depolarization can induce rhythmic activity in the coronary sinus (also in Purkinje fibers) and may be related to post-vagal tachycardia. Supported by USPHS grant #HL-14899.

**T-AM-Po130** CARDIAC LASER SCANNING OF SPREAD OF ELECTRICITY IN THE HEART.
S. Dillon & M. Morad, Dept. of Physiology, Univ. of Penna., Philadelphia, PA 19104

We developed a new method of scanning action potentials from a large number of sites on the heart (Dillon & Morad, Science 214:453,1981). A laser spot is rapidly (5μsec) positioned upon a heart stained with WW781 dye, by a dual axis acousto-optic deflector and the fluorescence from the entire specimen is transduced by a single photodiode amplifier (25μsec settle time). A computer system controls the sequence of laser spot positioning and correlates flourescence readings with physical locale. The accuracy of the technique was verified by simultaneous measurement of a 16 lead electrogram along with a 128 site scan of a thin Bullfrog atrial sheet. A scan of 128 sites is completed in 4 msec. Comparison of propagation maps from 128 sites on an atrial sheet, in 50μM $Ca^{2+}$, 1mM $Ca^{2+}$ and 1mM $Ca^{2+}$ plus $10^{-6}$M epinephrine, showed that motion artifact did not alter the conduction of the electrical signal. Scanning of intact Bullfrog heart gives a conduction velocity of 0.1 m/sec. Endocardial activation is faster than the epicardium with sinus rhythm. Elevation of $K^+$ reduces the conduction velocity and distorts the pattern of epicardial "breakout". We have also scanned a re-entrant tachycardia from the ventricular surface of an acutely ischemic cat heart. In the figure we show Bullfrog activation along with a scanned image of the heart.



**T-AM-Po131** INTRACELLULAR POTASSIUM AND CHLORIDE ACTIVITIES IN RABBIT ATRIO-VENTRICULAR NODE.
Clive Marc Baumgarten, Department of Physiology, Medical College of Virginia, Richmond, VA 23298.

Membrane potential ($E_m$) in the atrio-ventricular node (AVN) is significantly more positive than in adjacent myocardium and is only modestly affected by varying the superfusate potassium concentration between 2 and 20 mM. It has been suggested that the membrane is highly permeable to both potassium and chloride and that $E_m$ lies between $E_K$ and $E_{Cl}$, the equilibrium potentials for these ions. However, $E_K$ and $E_{Cl}$ are unknown. The intracellular activities of potassium ($a_K^i$) and chloride ($a_{Cl}^i$) were determined with ion-selective microelectrodes fabricated from Corning 477317 (K-ISE) and 477913 (Cl-ISE). N region of rabbit AVN was first delineated with 3 M KCl-filled microelectrodes using anatomical landmarks, action potential configuration and conduction time from the right atrium. After mapping, multiple impalements were made with K-ISE and Cl-ISE in cells exhibiting similar conduction times. Calculations were based on the average potentials obtained at the end of diastole. $E_m$ was -61 ± 4 mV in 5 mM potassium media. K-ISE potentials became 9 to 14 mV more positive on impalement indicating that $E_m$ was far positive to $E_K$. $a_K^i$ was 101 ± 8 mM, and $E_K$ was -87 ± 3 mV; these values are similar to those in other cardiac tissues. Cl-ISE potentials became 18 to 30 mV more negative on impalement. $a_{Cl}^i$ was 28 ± 3 mM, and $E_{Cl}$ was -38 ± 4 mV. Negligible Cl-ISE interference was assumed. The permeability ratio $P_{Cl}/P_K$ was estimated as 0.41 using the Constant Field equation. $P_{Na}$ and $P_{Ca}$ are very low at rest and thus were ignored. The data indicate that $E_m$ is between $E_K$ and $E_{Cl}$ and are consistent with the hypothesis that $E_m$ in AVN cells is determined by the electrochemical gradients for both of these ions.
Supported by NIH grant HL-24847 and the Elsie O. and Philip D. Sang Foundation.

**T-AM-Po132** BLOCKADE OF FAST INWARD SODIUM CURRENT BY QUATERNARY DERIVATIVES OF LIDOCAINE IN CANINE CARDIAC PURKINJE FIBERS. G.A. Gintant and B.F. Hoffman, Dept. of Pharmacology, Columbia University, New York, NY. 10032

We studied the interaction of the quaternary lidocaine derivatives QX-314 and QX-222 with canine cardiac sodium channels, using changes in the maximum rate of rise of the action potential upstroke (Vmax) to assess changes in fast inward sodium current. Both drugs caused tonic block (the reduction of Vmax which was not diminished by further slowing of stimulation). Tonic block could be established during equilibration of drug with non-stimulated preparations, as demonstrated by the reduction of Vmax of the first stimulated upstroke. Following periods of infrequent stimulation, rapid stimulation resulted in the exponential decline of Vmax during the stimulus train. We used the term use-dependent block to describe the reversible modulation of the reduction of Vmax following changes in the stimulation rate. The time constant characterizing the development of use-dependent block was reduced with increasing drug concentrations, and by increasing the stimulation rate. The development of use-dependent block was delayed by 100-200 msec depolarizing prepulses (than inactivated = 80% of available sodium channels) which terminated with the initiation of the upstroke. Following attainment of a steady use-dependent reduction of Vmax, similar depolarizing prepulses resulted in the recovery of Vmax revealed when the prepulses were discontinued. During slow stimulation, prolonged depolarizing prepulses (-50 to 0mV) which inactivated sodium channels during the diastolic interval did not reduce the Vmax of the subsequent upstroke. These results are consistent with the hypothesis that charged forms of local anesthetics interact with open cardiac sodium channels during the upstroke for the development of use-dependent block. In contrast, an upstroke is not required for the development of tonic block.

**T-AM-Po133** PERTURBATION OF SPONTANEOUS ACTIVITY OF EMBRYONIC CHICK HEART CELL AGGREGATES BY BRIEF DURATION CURRENT PULSES. M.R. Guevara[1], A. Shrier[1], J.R. Clay[2] and L. Glass[1] (Intr. by T.M.S. Chang[1]). [1]Dept. of Physiology, McGill University, Montréal, Québec, Canada H3G 1Y6; [2]Lab of Biophysics, NINCDS, MBL, Woods Hole, MA, USA 02543.

We have studied the effects of injecting brief current pulses into spontaneously beating heart cell aggregates. The aggregates were prepared from dissociated ventricular cells from 7 day old embryonic chick heart. Aggregates with diameters of 100-200 μm beat rhythmically in culture with interbeat intervals of 0.5 - 1 sec. Transmembrane voltage recording and current injection were carried out with a single microelectrode. We determined the response to injection of a single brief (20-40 msec) constant current pulse delivered at different phases of the spontaneous cycle. A depolarizing pulse delivered early in the cycle prolongs the duration of the perturbed cycle, while the same pulse delivered at a later phase shortens the cycle length. This biphasic behaviour is reversed in the case of hyperpolarizing pulses. For small pulse amplitudes, as the pulse is injected progressively later on in the cycle, there is a smooth transition from prolongation to shortening of the cycle length. For large pulse amplitudes, the transition is abrupt. Moreover, a pulse of critical amplitude injected at a critical phase of the cycle produces long prolongations of the order of several spontaneous cycle lengths. We have successfully simulated the effects of current pulses injected during the pacemaker depolarization using results obtained from voltage clamp measurements of background ($I_{bg}$) and time dependent ($I_{K2}$) pacemaker currents (Clay and Shrier, J. Physiol. 312, 471, 1981) and the fast inward sodium current ($I_{Na}$) (Ebihara et al., J. Gen. Physiol. 75, 437, 1980). The model generates biphasic responses as well as long prolongations similar to those experimentally observed. (Supported by the Canadian Heart Foundation).

**T-AM-Po134** POST-DRIVE MEMBRANE CURRENTS IN CANINE CARDIAC PURKINJE FIBERS. R.Falk and I.Cohen, Dept. of Physiology & Biophysics, SUNY at Stony Brook, Long Island, New York 11794

Post-drive currents were induced as previously described (Cohen, Falk, & Kline, 1981. Biophys. J. 33:281-288; Falk & Cohen, 1981. Biophys.J.33:36a). The dependence of the post-drive current on the frequency, duration, and membrane potential of the preceding period of overdrive was investigated. This dependence is correlated with the availability of the fast inward channel and slow inward channel. $Na^+$ entry through both of these channels presumably can contribute to the stimulation of the electrogenic Na/K pump, thereby inducing the post-drive current. Smaller currents, similar in time course to the post-drive current, can be induced by single depolarizing pulses of duration equal to that of the entire period of overdrive. This indicates that $Na^+$ loading through channels open in the steady state can also contribute to Na/K pump activation. Results of experiments which maximized $Na^+$ loading through the TTX-sensitive channel were most simply fit by a model assuming: (1)constant $Na^+$ loading/depolarizing pulse, throughout the overdrive, independent of pulse frequency for frequencies of 2 to 25 per second; (2)removal of intracellular $Na^+$ obeys first order kinetics (Gadsby, 1980; Eisner & Lederer, 1980). Results of experiments which included $Na^+$ loading through the slow inward channel necessitated modification of the assumption of a constant load independent of frequency. The model was modified to include the time and voltage dependence of slow inward channel gating. These models do not include either the expected effects of extracellular $K^+$ [$K^+$] fluctuations or possible indirect/direct contributions of electrogenic Na/Ca exchange.