W-AM-Po15 RESPONSE OF THE MACROPHAGE SURFACE TO CONCANAVALIN A: EFFECT OF SURFACE CROSS-LINKING ON THE ELECTROPHORETIC MOBILITY DISTRIBUTION. H. R. Petty and B. R. Ware, Harvard University, Cambridge, MA 02138

Electrophoretic light scattering (laser Doppler electrophoresis) has been employed to study the effects of concanavalin A (Con A) and succinyl-concanavalin A (S-Con A) on the electrophoretic mobility distribution of resident guinea-pig peritoneal macrophages. Con A, a tetrameric lectin, decreases slightly the mean mobility and increases the width of the electrophoretic mobility distribution of resident macrophages approximately three-fold. This effect can be abolished by α-methyl-D-mannoside, a hapten sugar of Con A. These effects were present in both low (0.010 M) and high (physiological, 0.15 M) ionic-strength media. Since lower ionic strengths correspond to a larger Debye screening distance, these data suggest that the alterations in the electrophoretic mobility distribution are not restricted to the outer portion of the glycocalyx. Succinyl-Con A, a dimeric derivative, was found to have no effect on the mobility distribution. However, the mean mobility decreased and the width increased over four-fold when S-Con A-treated macrophages were exposed to anti-Con A. These observations indicate that cross-linking of Con A receptors is an important process in the electrokinetic alterations of the macrophage surface. Other workers have noted Con A patch formation on the resident macrophage surface is followed by vesicle formation and, ultimately, in the formation of large cytoplasmic vacuoles; this process occurs with Con A but not S-Con A. The segregation of various components such as lipids, transport functions, Fc receptors, and lectin receptors has also been noted. Our data indicate that there is a non-random partitioning of surface charge density during the endocytosis of Con A patches; the width of the mobility distribution indicates that macrophages are heterogeneous in this aspect of their response to Con A.

W-AM-Po16 FREEZE-FRACTURE STUDY OF RAPIDLY FROZEN FROG RETINAL ROD OUTER SEGMENTS. J.M. Corless* and M.J. Costello, Dept. of Anatomy, Duke Univ. Medical Center, Durham, NC 27710.

Suspensions of isolated frog rod outer segments (ROS) have been well preserved in the absence of fixatives or cryoprotectants by ultra-rapid freezing of thin samples (about 10μm thick and 0.1μl total volume) sanwiched between copper sheets and plunged into liquid coolants (see J. Microsc. 112: 103-113, 1978). No evidence of ice crystal damage is present and direct measurements using thermocouples embedded in these samples indicate that cooling rates are in excess of 5000°C/s (see J. Microsc. 112: 17-37, 1978). ROS thus preserved reveal rough (PF) fracture faces of disk membranes containing predominantly an irregular pattern of interconnecting ridges, and relatively smooth (EF) fracture faces containing a similar distribution of crevices. These fracture faces do not display typical intramembrane particles or pits and are in general more complementary than those found in conventionally frozen ROS. In some regions of EF fracture faces, two-dimensional lattices have been observed. In such regions, the dominant appearance is a series of parallel rows, spaced about 50-60Å apart. Along each row, the repeat period, when detectable, is about 75Å. An unusual fracture pattern is seen in some longitudinal fractures of ROS in which the entire disk has been pulled out, leaving a scalloped surface in the remaining cytoplasm. Another type of regularity is observed for paracrystalline inclusions embedded within the disk membrane array. The inclusions appear to be segregations of lipid or glycolipid forming domains of variable morphology. In some regions, the complex fracture patterns are very similar to those observed for hexagonally packed lipid cylinders.

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W-AM-Po17 FOURIER TRANSFORM INFRARED STUDIES OF BIOLOGICAL MEMBRANES. D.G. Cameron*, H.L. Casal* and H.H. Mantsch,
Division of Chemistry, National Research Council Canada, Ottawa, K1A OR6.

Fourier transform infrared spectroscopy has been applied to investigate the spectra of a variety of biological membranes. Changes induced by external factors such as temperature or addition of perturbing molecules into the membrane, have been monitored for a series of deuterated Acholeplasma laidlawii membranes. Perdeuterated palmitic acid was incorporated biosynthetically into the lipids of the plasma membrane of Acholeplasma laidlawii and the temperature-induced structural rearrangement of the endogenous lipids monitored via their C-D vibrational modes. Changes in infrared parameters were studied between 0 and 50°C and contrasted with those occuring in the model membrane system of 1,2-diperdeuteropalmitoyl-sn-glycero-3-phosphocholine. The phase transition of the biomembrane occurs over a 20°C range with the temperature of the maximum rate of change of absorbance coinciding with that of the sharp phase transition of the model membrane.

W-AM-Po18 REGULATION OF ZOOSPORE ENCYSTMENT: STUDIES ON THE PLASMA MEMBRANE OF THE AQUATIC FUNGUS *Blastocladiella emersonii*. K. S. Leonards and A. Haug*, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

Zoospores of *Blastocladiella emersonii* are suited for studying the role of membranes in morphogenesis, separate from those involving protein or RNA synthesis. The ability of zoospores to encyst is controlled by the ionic environment, specifically K^+ and Ca^{++} ion concentrations. The *in vivo* influence of Ca^{++} ions on the physical properties of zoospore membranes was investigated by EPR spectroscopy using the spin labels 5-nitroxide stearate (5NS) and 2,2,6,6-tetramethylpiperdine-1-oxyl (TEMPO). The results indicated three 'breaks' in the plots of the hyperfine splitting parameter, order parameter, and partition ratio versus temperature. The first (5-7°C) and third (34-36°C) breaks are independent of ion concentration. The 'break point' in between, however, can be shifted by Ca^{++}. In the presence of buffer only (MOPS), 1-10 mM $MgCl_2$ or 50 mM KCl, this 'break' is at 10-12°c. However with increas-Ca^{++} ion concentrations this 'break' is shifted to progressively higher temperatures, until at 10 mM $CaCl_2$ it occurs at 22-23°C., i.e. growth temperature 22°C. These findings correlate well with previous physiological studies which demonstrated that 10 mM Ca^{++} inhibits encystment of zoospores at growth temperature. Experiments with vesicles made from whole cell lipid extracts indicate only two 'break points' (1-2°C and 30-31°C, respectively), but do not show a corresponding middle 'break point'. Addition of Ca^{++} to the vesicle preparation results in the rigidification of the membrane over the entire temperature range, but no change in the two 'break point' temperatures nor appearance of an in between 'break point'. These results suggest that Ca^{++} may not be affecting the bulk properties of the zoospore membrane, but instead local properties observed with spin labels. Supported by US-DOE contract EY-76-C-02-1338.

W-AM-Po19 ELECTRON SPIN RESONANCE SPECTROSCOPY STUDIES OF THE PLASMA MEMBRANE OF SODIUM CHLORIDE ADAPTED *DUNALIELLA PRIMOLECTA*.
D. R. B. Fontana and A. Haug*, Department of Biophysics and MSU/DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824.

Dunaliella primolecta is a wall-less green alga which can be adapted to grow in external NaCl concentrations ranging from 0.25% to 29% (0.04 M to 4.96 M). It appears that the plasma membrane of this organism plays an important role in the salt adaptation process. The plasma membranes of algae grown at different NaCl concentrations were studied in vivo with electron spin resonance spectroscopy. The fatty acid spin probes five- and sixteen-nitroxide stearate (5NS,16NS) were employed. Reduction by ascorbate was used to confirm the position of the label in the plasma membrane. When possible, the order parameter, correlation time and $2T_{\parallel}$ were calculated. When these values were plotted as a function of temperature, four discontinuities in slope were observed. Indirect evidence indicates that the highest (42°C) and the lowest (5°C) discontinuities correspond to the bulk lipid transition. These breaks appear not to vary as a function of the NaCl concentration in the growth medium. One of the remaining breaks does vary as a function of external NaCl. The values of the calculated parameters indicate that the plasma membrane of *D. primolecta* is a very rigid one whose physical properties can be affected by the concentration of NaCl in the medium.
Supported by US-DOE contract number EY-76-C-02-1338.

W-AM-Po20 LIPOSOME-CELL INTERACTIONS: STUDIES OF LIPID TRANSFER USING ISOTOPICALLY ASYMMETRIC VESICLES. Alexander Sandra* and Richard E. Pagano, Carnegie Institution of Washington, Dept. of Embryology, 115 W. University Pkwy., Baltimore, Md. 21210

Phospholipid transfer between small unilamellar vesicles and Chinese hamster fibroblasts, a process in which cellular uptake of vesicle lipids without concomitant uptake of vesicle contents occurs, was studied. Isotopically asymmetric vesicles were formed by incubation of ^3H-labeled dioleoyl phosphatidylcholine or ^3H-labeled dioleoyl phosphatidylethanolamine-containing vesicles with appropriate phospholipid exchange proteins and unlabeled acceptor membranes. Isolated vesicles were then incubated with cell suspensions at 2°C and cellular uptake of radioactive lipid was determined. Uptake of exogenous lipids was found to be directly proportional to the amount of radioactivity present in the outer (but not inner) leaflet of the vesicles, thus demonstrating that only the outer monolayer lipids become cell associated during vesicle-cell lipid transfer. Immediately following incubation of cells with ^3H-labeled phosphatidylethanolamine-containing vesicles at 2°C, more than 80% of the exogenously supplied lipid was converted to trinitrophenyl phosphatidylethanolamine when the cells were reacted with trinitrobenzene sulfonic acid (TNBS) under non-penetrating conditions. This result demonstrates that vesicle lipids are introduced preferentially into the outer leaflet of the cell plasma membrane by the lipid transfer process. Spectrophotometric determination of the total amount of phosphatidylethanolamine accessible to TNBS labeling before and after incubation of cells with vesicles revealed that lipid transfer is not accompanied by a net change in cell surface phosphatidylethanolamine, suggesting that the transfer of lipids between vesicles and cells actually represents a one-for-one exchange. This exchange process is phospholipid species specific and sensitive to pretreatment of cells with trypsin. Supported by GM22942

W-AM-Po21 USE OF HYPERTHERMIA FOR SELECTIVE LOCALIZATION OF LIPOSOMAL METHOTREXATE IN TUMORS. J.N. Weinstein[1], R.L. Magin*[1], D.S. Zaharko*[1], M.B. Yatvin[2], and R. Blumenthal[1]; NCI, NIH, Bethesda, MD 20014[1] and University of Wisconsin, Madison, WI 53706[2].

Use of liposomes as pharmacological capsules has been limited by the inability to direct them selectively to particular cells or anatomical sites. We have taken a new approach to the problem based on the well-known propensity of liposomes to release small solutes at temperatures near the phase transition. Small sonicated vesicles of 3:1 (molar) dipalmitoyl phosphatidylcholine (DPPC)-distearoyl phosphatidylcholine (DSPC) were found to leak carboxyfluorescein dye maximally in the range 42.5 to 44.5°C during temperature scans. The release process could be accelerated by increasing the scan rate, by substituting multilamellar for small unilamellar vesicles, and, most dramatically, by including serum components in the medium. In a model system in vitro, neomycin released from DPPC-DSPC vesicles near the transition temperature effectively inhibited protein synthesis and colony formation by E. coli.

When DPPC-DSPC liposomes containing ^{3}H-methotrexate (^{3}H-MTX) were injected intravenously in mice carrying subcutaneous Lewis lung tumors, local microwave heating of the tumors to 42° doubled the rate at which MTX was cleared from the blood, suggesting a release of drug from the vesicles in that small fraction of blood passing through the heated area in each circulation. Heated tumors accumulated on average 4.3 times as much MTX as unheated control tumors in the same animals. Two findings indicated that the accumulated MTX was intracellular and bound to its target enzyme, dihydrofolate reductase: (i) a large proportion of the MTX remained in the tumor even after 20 hours, (ii) its accumulation was largely blocked by competition with unlabelled free MTX. High pressure liquid chromatography showed virtually all of the ^{3}H counts in the tumors to be from intact MTX.

W-AM-Po22 RNase SENSITIVE SITES ON THE SURFACE OF THE S-180 MOUSE TUMOR DISAPPEAR AFTER TREATMENT WITH THE ANTICANCER DRUG CISPLATIN. D.A. Juckett, B. Rosenberg*, Biophysics Dept. Michigan State University, East Lansing, MI 48824.

Through the use of whole cell electrophoresis, it was shown that the S-180 mouse ascitic tumor cell has RNase sensitive sites on its surface. These sites are detected by the change in the net negative surface charge after enzyme treatment. Approximately 22% of the net negative charge is due to these sites and is unchanged after repeated washings. The mobility of the tumor is 1.37 μ/sec/V/cm and after treatment with RNase is 1.07. As suggested by others[1,2], we believe these sites represent surface RNA. After treatment of the mice with cis-dichlorodiammineplatinum(II)(cisplatin), the tumor cells no longer exhibit RNase sensitive sites and the mobility of these treated cells is 1.09, which is the same as the RNase treated tumor cells which were not exposed to cisplatin. Pt-thymine blue, a positively charged oligomer, at concentrations of 10 μg/ml also binds to these sites and negates the charge in the controls but shows no binding in the cisplatin treated mice. The Pt-blues interact strongly with nucleic acids. It is possible that these nucleic acids are important in the body response to the tumor and that one effect of cisplatin therapy is to enhance that response. These possibilities will be presented. (1) J. Woo & D.B. Cater, Biochem. J. 128 (1972) 1273-84; (2) E. Mayhew & L. Weiss, Expt. Cell Res. 50 (1968) 441-453.

W-AM-Po23 TRANSFER OF LIPOSOME CONTENTS TO LYMPHOCYTES: STUDIES OF MECHANISM. R. Blumenthal, E. Ralston*, P. Dragsten*, and J.N. Weinstein, LTB, NCI, NIH, Bethesda, MD 20014.

Transfer of carboxyfluorescein (CF) from small unilamellar dioleoyl phosphatidylcholine vesicles to human peripheral blood lymphocytes is a complex process consisting of at least two kinetic components. One component saturates as a function of concentration and can be abolished almost completely by fetal calf serum (FCS) (Blumenthal, et al., PNAS 74:5603, 1977); the other, which is much slower, does not saturate in the range of vesicle concentrations studied (up to 3 mM lipid). Saturable transfer was also found with human red cell ghosts and with a lymphoid tumor line. In further studies on the lymphocytes, we found that the non-saturable component of liposome-mediated CF transfer and the entry of free CF into the cell had similar rates. They were also affected to approximately the same extent by a combination of sodium azide and 2-deoxy-D-glucose (2-dG) (55% inhibition). Furthermore, free ^{3}H-inulin was incorporated into the cells at almost the same rate as CF and was inhibited to the same extent by azide and 2-dG. The observation that the non-saturable uptake from liposomes was commensurate with that of free CF and free inulin suggests pinocytosis as a mechanism. When liposomes containing the fluorescent lipid analogue 3,3'-dioctadecylindocarbocyanine iodide were incubated with lymphocytes, the label did not spread out over the cell membrane, and there was no recovery after photobleaching. A model of transfer in which liposome contents leak at the cell surface and are subsequently taken into the cell is ruled out by calculations showing that this mode of entry would lead to far too low a rate of liposome-mediated transfer. Moreover, the presence of cells did not affect leakage of liposome contents. Interpretation of these findings remains highly speculative but we propose for further study the possibility that the saturable transfer occurs by direct passage of liposome contents across the two closely apposed membranes of liposome and cell.

W-AM-Po24 ENTRAPPMENT OF PROTEINS OF VARIOUS MOLECULAR WEIGHTS INTO PHOSPHATDYLCHOLINE VESICLES. Gary Adrian* and Leaf Huang (Intr. by S. W. Riggsby), Dept. of Biochemistry, University of Tenn., Knoxville, TN 37916.

Phospholipid vesticles have been used as vehicles to deliver macromolecules into cells. In this study, the entrappment of globular proteins of various molecular weight (m.w.) was investigated in different types of phospholipid vesicles. These vesicles were generated in a mixture of I^{125}-proteins of various m.w. The trapped protein is separated by two consecutive gel filtrations, Sepherose 4B and Sephadex G-100. The entrapped protein is analyzed by SDS-PAGE on Slab gels and autoradiography. Entrappment of the proteins was demonstrated by their resistance to trypsin and chymotrysin digestion. The relative amount of each entrapped protein was then compared to that of the original protein solution. In small unilamellar vesicles (SUV) prepared by ethonol injection-method (Batzri and Korn, BBA 298, (1973) 1015), proteins of m.w. less than 40,000 dalton could be trapped with the same efficiency as sucrose; whereas proteins of m.w. greater than 40,000 dalton showed progressive decrease in the trapping efficiency. In hand shaken multilamellar vesicles and large unilamellar vesicles (Deamer and Bangham, BBA 443 (1976) 629), proteins of m.w. up to 120,000 dalton showed identical trapping efficiency as sucrose. The apparent molecular weight cutoff in the trapping efficiency of SUV's is much smaller than the therotical values calculated from the vesicle internal volume. Therotical considerations for the apparent discrepency will be discussed. Supported by NIH Grant GM23473.

W-AM-Po25 EFFECTS OF PHOTODYNAMIC ACTION ON BIOLOGICAL MEMBRANES. G.E. Cohn, J.M. Collins* and J.E. Clark* Biophysics Laboratory, Physics Department, Illinois Institute of Technology, Chicago, IL 60616

Photodynamic irradiation with visible light in the presence of eosin Y modifies the properties of lipid regions of yeast cells while inactivating the cells. When the Saccharomyces cerevisiae strain Y55 was inactivated and spin labeled with the fatty acid probe 12NS, ESR spectra showed that the labels divided into two populations which appeared to correspond to a polar and hydrocarbon location while only the hydrocarbon signal appeared before irradiation. Upon addition of 1.0 M $NiCl_2$ following irradiation, the polar signal was quenched and the hydrocarbon component was left intact. Spin reduction occurred irreversibly for unirradiated Y55 if the temperature exceeded 30°C. For inactivated Y55 the polar signal was predominant at 0°, but above 30°C the hydrocarbon component was predominant both without and with $NiCl_2$. No spin reduction occurred for inactivated cells at temperatures from 0°C up to 40°C. Cells labeled with the hydrocarbon probe 7N14 showed no polar contribution to the spectrum either before or after irradiation, nor was rapid spin reduction observed in either case over the same temperature range. A steady increase in spin label tumbling time τ_o was observed when Asolection lipsomes were photodynamically irradiated and labeled with the methyl ester of 12NS. τ_o varied from 1.5 ns initially to 3 ns after treatment but all spectra had hyperfine splittings characteristic of a non-polar environment suggesting damage to the lipid bilayer. These data indicate that eosinsensitized photodynamic irradiation significantly alters the physical properties and function of membrane regions. (Supported by Research Corporation Cottrell Research Grant No. 7250 and by U.S. Department of Energy Contract EY-76-02-2217).

W-AM-Po26 ASYMMETRY OF PHOSPHOLIPID BIOSYNTHESIS IN RAT LIVER MICROSOMES. E. A. Dawidowicz and J. R. Sawyer*, Biophysical Laboratory, Harvard Medical School, Boston, MA 02115

Rat liver microsomes have been attached to glass beads which possess a positive charge using the principles described by Jacobson and Branton (Science (1977) 195, 302). After lysis and sonication, 80% of the glucose-6-phosphatase activity is lost from the membrane following digestion with chymotrypsin, whereas the NADPH-cytochrome c reductase activity remains unaffected. Since the opposite results have been reported for proteolysis of intact microsomes, we conclude that the microsomal membranes attached to the beads as described above have an inside-out orientation. Using this preparation, it was found that greater than 80% of both the diacylglycerol cholinephosphotransferase and the corresponding ethanolaminephosphotransferase activities were unaffected by chymotrypsin digestion. Coleman and Bell (J. Cell. Biol. (1978) 76, 245) have shown that greater than 90% of these enzyme activities, which are responsible for the synthesis of phosphatidylcholine and phosphatidylethanolamine, are removed by proteolysis of intact microsomes. Our results therefore confirm the asymmetry of phospholipid biosynthesis in microsomes. (Supported by NIH Grant GM22175).

W-AM-Po27 CHANGES IN SURFACE PROPERTIES OF DEVELOPING ZOOSPORES OF *BLASTOCLADIELLA EMERSONII*: THE BINDING OF CONCANAVALIN A. by C.J.Jen and A.Haug*. Department of Biophysics and MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824.

Zoospores of *Blastocladiella emersonii*, an aquatic unicellular fungus, undergo synchronous encystment which can be controlled by varying the external cation concentration. During the encystment process surface alterations take place. Binding of concanavalin A to the surface was used to derive information about the state of the cell surface during development. Fluorescence and electron microscopy, and radioactive labelling were employed. Synchronously induced zoospores revealed a sharpening of the fluorescence intensity distribution which was broad in non-induced cells. The zoospore surface contained $3x10^7$ concanavalin binding sites per cell which were randomly distributed and closely attached to the cellular surface. The affinity constants ranged from $2x10^{-8}$M to $2.5x10^{-6}$M, while the Scatchard plot suggested heterogeneous binding. Further developed cells, *i.e.*, round cells and germlings, contained 1.5 to $2.0x10^7$ concanavalin receptor sites which comprised mostly loosely associated patches protruding into the extracellular region. The affinity constants increased appreciably as compared to those measured at the zoospore stage. In conclusion, specific concanavalin A binding characteristics appear to correlate with the respective developmental stage of the zoospore. Supported by US-DOE contract No. EY-76-C-02-1338.

W-AM-Po28 ADENOSINE RECEPTORS IN FROG SINUS VENOSUS MYOCYTES. H. Criss Hartzell* (Intr. by D. P. Valenzeno), Department of Anatomy, Emory University School of Medicine, Atlanta, Ga. 30322.

Adenosine receptors were studied in the isolated frog sinus venosus by intracellular recording and application of adenine compounds by iontophoresis and bath application. Adenosine at concentrations as low as 10^{-7}M produced hyperpolarizations in sinus myocytes. The apparent dissociation constant of adenosine binding to its receptor was estimated from dose-response curves to be 2μM. Adenosine and adenine nucleotides were virtually equipotent in their action. Other purine and pyrimidine nucleosides were at least 1000-fold less potent than adenosine in producing a hyperpolarization. Three lines of evidence show that adenosine acts directly upon surface receptors in the membrane of the muscle cells. Analogs of adenosine which do not enter cells are potent agonists of the receptor. An adenyl oligonucleotide too large to permeate the cell membrane was also a potent agonist. Drugs (such as dipyridamole and hydroxy-nitrobenzyl thioguanosine) which block adenosine transport potentiate the response of sinus myocytes to adenosine. Adenosine action is blocked competitively by theophylline (K_i = 5μM), but atropine has no effect.

W-AM-Po29 INTERACTION OF NICOTINIC RECEPTOR AFFINITY REAGENTS WITH CENTRAL NERVOUS SYSTEM α-BUNGAROTOXIN-BINDING ENTITIES. Ronald J. Lukas and Edward L. Bennett*, Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720.

In the absence of direct physiological or pharmacological evidence that α-bungarotoxin (α-Bgt) acts toward central nervous system nicotinic acetylcholine receptors (nAChR) as an antagonist, establishment of identity between toxin receptors and physiologically important nAChR remains equivocal despite a wide range of supporting histological and biochemical data. We now report that two affinity reagents designed to label peripheral nAChR also label the α-Bgt-binding entity from rat brain. Bromoacetylcholine (BAC) and 4-(N-maleimido) benzyl trimethylammonium (MBTA), when present at concentrations of 50 and 0.2 μM, respectively, alkylate free sulfhydryl group(s) in DTT-reduced brain membranes to the extent that one-half of specific tritiated-α-Bgt binding is prevented. In contrast, concentrations of BAC and MBTA of 5.0 and 0.2 mM, respectively, are necessary to prevent one-half toxin binding to DTT-reduced membranes which have been treated with DTNB or NEM prior to exposure to BAC or MBTA. When affinity reagents are simultaneously incubated with toxin and 'native' membranes, toxin binding is blocked in a biphasic fashion. This presumably reflects preexistence of a mixed population of reduced and non-reduced receptors, each of which exhibits a characteristic affinity for BAC/MBTA. These data strongly suggest that central α-Bgt receptors and physiologically important central nAChR are identical.

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W-AM-Po30 CHOLINERGIC AGONIST INDUCED VOLTAGE DEPENDANT CURRENTS IN VOLTAGE CLAMPED OOCYTES OF XENOPUS LAEVIS. C. Franco-Hidalgo* and F. Sachs. Department of Pharmacology, SUNY at Buffalo, Buffalo, New York 14214.

Xenopus oocytes possess three distinct conductance regions: a low conductance region between about 0 mV and -55 mV, which we term the passive component. At positive potentials, a large outward current develops with a sharp threshold. At potentials negative to -55 mV another high conductance region appears which carries inward current. The threshold for the high conductance regions is changed by cholinergic agonists, particularly muscarinic agonists. The threshold on the positive side is made more negative and the threshold on the negative side is made more positive. The "passive" region decreases its conductance only slightly (~ 2X) in response to agonists (acetylcholine, carbamylcholine, bethanechol). The high conductance regions, however, involve changes of more than an order of magnitude in response to agonists. The agonist induced response is blocked by atropine at 10^{-6} M. Assays of ^{125}I α bungarotoxin binding to ooryte surfaces and broken cells have shown no specific toxin binding sites. Comparison of the physical size of the egg and the membrane capacitance give a specific capacity of about 1 $\mu F/cm^2$, implying no infoldings. This response may be analogous to the response of cardiac calcium conductance as affected by catecholamines. Supported in part by funds from grants HL21294 and NS13194.

W-AM-Po31 LATERAL DIFFUSION OF MEMBRANE ANTIGENS AND A LIPID PROBE IN LYMPHOCYTES. P. Dragsten*, J. Schlessinger*, P. Henkart*, J.N. Weinstein, and R. Blumenthal (Intr. by A. Babcock), NCI, NIH, Bethesda, Md. 20014.

Fluorescence photobleaching recovery measurements were used to determine lateral diffusion coefficients of surface immunoglobulin (sIg--detected with rhodamine-goat anti-mouse IgG), Thy 1 antigen (detected with rhodamine-rabbit anti-mouse brain), and a lipid probe (3,3'-dioctadecylindocarbocyanine iodide) in the plasma membrane of mouse spleen lymphocytes. Sixty to eighty precent of the detectable sIg and Thy 1 antigen are free to move in the plane of the membrane when labeled with the monovalent Fab antibodies, with diffusion constants of about $3x10^{-10}$ cm^2/sec. sIg diffusion could be restricted by crosslinking with appropriate concentrations of bivalent (IgG) anti-immunoglobulin (in the presence of azide to inhibit capping). The extent of immobilization of sIg was dependent on the concentration of anti-Ig used, and correlated well with the frequency of cap formation in the absence of azide. Immobilization of Thy 1 was not readily observed with the IgG rabbit anti-mouse brain antiserum, and the antigen did not cap. Addition of a second layer of crosslinking antibody (goat anti-rabbit IgG) did produce both capping and immobilization. It appears from these results that, under crosslinking conditions sufficient to induce cap formation, sIg and Thy 1 antigen are essentially immobile with respect to diffusion over distances of a micron or greater. The fluorescent lipid probe gave $D=(1.5\pm.3)x10^{-8}$ cm^2/sec, with essentially 100% of the probe free to diffuse in the plane of the membrane. We also measured the lipid probe's diffusion coefficient on individually identified T and B lymphocytes by double staining experiments using fluorescein-conjugated anti-mouse Ig or rabbit anti-mouse brain. No differences in D were observed between these two cell populations.

W-AM-Po32 ABSTRACT TRANSFERRED

W-AM-Po33 THE INTERACTION BETWEEN SURFACE IMMUNOGLOBULIN RECEPTORS AND MITOGENS IN B LYMPHOCYTE ACTIVATION. A. Rosenspire, State University of New York at Buffalo, Buffalo, New York 14214.

A model scheme is presented to show how ligand binding to one type of membrane receptor may modulate the activity of a chemically distinct surface receptor to an entirely different ligand. Specifically the behavior of the B lymphocyte and its response to surface immunoglobulin-antigen binding and to mitogen binding interactions has been modeled. The model is based upon the concept of an allosteric transmembrane mitogen receptor that is assumed to mediate signal transduction via a single second messenger. The mitogen receptor is also capable of interacting with surface immunoglobulin, so that immunoglobulin-antigen interactions may be capable of modulating the receptor response to the mitogen.

We have performed a series of in vitro experiments utilizing the T-independent antigen TNP-LPS and the mitogen lipopolysaccharide with mouse spleen cells, and show that in this system at least, the experimental results can be interpreted in a systemic manner, in qualitative agreement with the predictions of the model. These same experiments cannot easily be reconciled with previous models of B cell activation.

W-AM-Po34 SAXITOXIN BINDING TO WHOLE FROG HEART AND FROG HEART HOMOGENATES M. Wong* and L. Barr, Dept. Biochemistry, U. of Va., Charlottesville, Va. 22908; Dept. Physiology and Biophysics, U. of Illinois, Urbana, Ill. 61801.

Tritium labelled saxitoxin (STX) binding to whole frog heart and frog heart homogenates was measured. Whole atria STX binding had a maximum saturable capacity, M=6.4±.3 f-mole/mg wet weight, inhibited by 10 micromolar unlabelled tetrodotoxin (TTX). Homogenized atria STX binding (assayed by equilibrium dialysis) had a maximum saturable capacity, M=126±2 f-mole/mg wet weight, not inhibited by 10 micromolar unlabelled TTX. Ultracentrifugation studies showed that the (TTX-independent)-STX binding site is soluble, remaining in the supernatant from a 100K x g, 120' spin. Binding to the sarcolemma membrane vesicle fraction, at the interface above a 1.15 density step sucrose gradient, gave a maximum saturable component of about 1 p-mole/mg protein, consistent with the number of STX binding sites per mg membrane protein calculated in whole tissue. The 20 fold greater STX binding in homogenate over whole tissue indicates that the soluble (TTX-independent)-STX binding sites are located in the cell interior, and are exposed upon tissue homogenization. The level of soluble (TTX-independent)-STX binding varies between Northern and Southern Rana pipiens and between atria, bulbous arteriosis, and ventricle. STX was a gift from Dr. E.J. Schantz. Tritiated STX was a gift from Dr. G. Strichartz.

W-AM-Po35 SYNTHESIS AND FLUORESCENCE PROPERTIES OF N-DANSYL-MET-ENKEPHALIN. R.G. Canada, M.A. El-Bayoumi, J.I. Johnson and E.J. McGroarty, Biophysics Dept., Michigan State Univ., East Lansing, MI 48823.

Fluorescently labelled enkephalins offers a useful technique to study opiate receptor sites. We have successfully labelled met-enkephalin with a dansyl group at the amino terminal. N-dansyl-met-enkephalin exhibits a fluorescence maximum at 528 nm in ethanol. It is known that the emission of the dansyl group undergoes a large red shift when dansyl chloride is changed to dansyl amides (e.g., N-dansyl-amino acids). The fluorescence intensity and maximum are sensitive to the dielectric constant of the medium. Details regarding the preparation and purification as well as the fluorescence properties of N-dansyl-met-enkephalin will be discussed. We have also obtained preliminary data regarding the interaction of labelled enkephalin with cultured amygdaloid neurons. The utility of using fluorescently labelled enkephalins to study opiate receptor sites will be presented.

W-AM-Po36 FURTHER DEVELOPMENT OF A MODEL FOR ELECTRICAL TRANSMISSION BETWEEN MYOCARDIAL CELLS NOT CONNECTED BY LOW-RESISTANCE PATHWAYS. J.E. Mann, Jr.* and N. Sperelakis, Departments of Applied Mathematics and Physiology, University of Virginia, Charlottesville, VA 22903.

Sperelakis and Mann (1977) presented a new model that would allow electrical transmission to occur between contiguous myocardial cells, but without the requirement of low-resistance connections between cells. In their model, they analyzed the electric field that would develop in the narrow cleft between two contiguous cells during an action potential in one cell, with the assumption that the prejunctional membrane was an excitable membrane. It was shown that when the pre-membrane fired, the narrow cleft between the cells became negative with respect to ground (fluid surrounding the cells), and this negative cleft potential acted to depolarize the post-membrane to its threshold and the post-cell. The inner surface of the post-membrane remained at nearly constant potential with respect to ground, and virtually no local-circuit current flowed through the post-cell. Because cancellation of the cleft potential occurred if the surface membrane near the pre-membrane fired simultaneously with the pre-membrane, it was required for the successful transfer of excitation that the pre-membrane fire a fraction of a millisecond before the immediately adjacent surface membrane. This was made possible by lowering the threshold of the junctional membranes slightly. This model was analyzed only for the steady-state condition. In this subsequent study, the effect of the membrane capacitances on the response of the model was analyzed. The calculations indicate that it is somewhat easier to achieve transmission when capacitative effects are included. Transmission was facilitated when the junctional capacitance (time constant) was lowered by a factor of several fold, relative to the surface membrane capacitance. Thus, the electric field model provides an alternative means of cell-to-cell propagation between myocardial cells which is electrical in nature but does not require the presence of low-resistance connections between cells.

W-AM-Po37 EVIDENCE FOR A FIXED CHARGE IN THE NEXUS. P. R. Brink and M. M. Dewey, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Fluorescein permeability to the nexus is 5×10^{-5} cm/sec (Brink and Dewey, J.G.P., 1978). Aminofluorescein has a much lower permeability (4×10^{-7}). 6-carboxyfluorescein has a permeability of 2×10^{-6}. The widest dimension of all three molecules is 9.5 Å. On the basis of size all three molecules should have similar permeabilities. The molecule weights vary from 333 for fluorescein to 376 for carboxyfluorescein. The nexal membrane permeability to dichlorofluorescein is 3.8×10^{-5} cm/sec (mol. wt. = 401) therefore molecular weight must not be the reason for the lower permeability. The extra positive charge of aminofluorescein and negative charge of 6-carboxyfluorescein are probably responsible for their depressed permeabilities if there is a fixed charge in the "aqueous channel". In addition, iontophoresis of aminofluorescein into a cell followed by fluorescein causes the fluorescein permeability to be lowered by 60 to 90% (7×10^{-6}). In similar experiments where 6-carboxyfluorescein was pre-injected followed by fluorescein, no decrease in fluorescein permeability occurred. This implies an interaction of aminofluorescein and the fixed negative charge of the "channel".

Lipid solubility could also be a factor in nexal membrane permeability. For this reason the partition coefficients were determined for a number of dye molecules whose nexal membrane permeabilities were measured (Levitan, PNAS, 1977). There is an inverse relationship between a molecule's nexal permeability and lipid solubility and a proportional one for membrane permeability and lipid solubility.

Supported by USPHS AM 18750.

W-AM-Po38 SPREADING CILIARY ARREST IN GILL EPITHELIA: RELATION TO Ca^{2+} INFLUX. W. Reed* and P. Satir (Intr. by S. Seifter). Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461.

When active, the lateral (L) cilia of the gill of freshwater mussels (e.g. Elliptio) beat with metachronal waves (MW). We have previously demonstrated that in appropriate solutions, local laser microinjury to a few cells induces temporary ciliary arrest that spreads bidirectionally outward from the lesion for 30-60 cell lengths. Arrest is characterized by the loss of metachronal waves and usually lasts for tens of seconds, during which time the epithelium apparently remains electronically coupled (Satir et al. Nature 263: 520-521, 1976). We have analyzed these experiments further in two ways. (1) The time course permits us to use quick fixation with 1% OsO_4 to study the morphology of arrested vs. active cilia during recovery. Arrested cilia all lie in the unique "hands up" position that also characterizes cilia arrested by application of Ca^{2+} ionophore A23187 in the presence of Ca^{2+}. Some axonemes show ciliary collapse, but this is not universal. The transition between arrested and MW cilia is abrupt and often coincides with a cell border. (2) We have defined more completely ionic conditions under which arrest is not induced. Arrest is reversibly inhibited in the presence of Ca^{2+}-EGTA buffers with free $Ca^{2+} < 10^{-8}$ M. It is also inhibited in the presence of normal Ca^{2+} if a Ca^{2+} channel blocking agent such as La^{3+} (1 mM) is included in the bathing medium. These data support our hypothesis that microinjury induces an electronically spread depolarization followed by an inward Ca^{2+} flux along the filament that increases cytoplasmic free Ca^{2+} to arrest the cilium.

Supported by USPHS 22560

W-AM-Po39 INTRACELLULAR pH, INTRACELLULAR Ca^{2+}, AND CELL-TO-CELL COUPLING. B. Rose and R. Rick,*[1] Dept. Physiol. & Biophys., Univ. Miami, Miami, FL 33101.

Intracellular pH (pH_i) and intracellular Ca ($[Ca^{2+}]_i$) were monitored in Chironomus-thummi salivary gland cells under various conditions of experimentally induced uncoupling in an effort to determine the interrelationship between $[Ca^{2+}]_i$ and pH_i on one hand, and cell-to-cell coupling on the other hand. pH_i was measured with Thomas-type recessed-tip microelectrodes;[2] aequorin was used for detection of $[Ca^{2+}]_i$ changes, and cell-to-cell coupling was measured electrically. We found uncoupling to be correlated with an increase in $[Ca^{2+}]_i$ by treatment with NaCN, DNP, or A23187—all of which effected little change in pH_i. Likewise, Ca^{2+} injections avoiding pH_i changes led to uncoupling. Cell alkalinization (with NH_4^+, e.g.) also increased $[Ca^{2+}]_i$ and uncoupled cells. Cell acidification by 100% CO_2 could lead to an elevation of $[Ca^{2+}]_i$, and uncoupled cells; H^+ injection increased intracellular Ca^{2+} and caused uncoupling. We conclude that a decrease in pH_i is not necessary to effect uncoupling, while an increase in $[Ca^{2+}]_i$ is sufficient.

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[2]Thomas, R., 1974. J. PHysiol. 238:149-180

W-AM-Po40 CHANGES IN JUNCTIONAL CELL-TO-CELL COMMUNICATION DURING DEVELOPMENT. R.W. Wagner and W.R. Loewenstein, Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33101.

These experiments test the hypothesis of a programmed, selective transmission of cell-to-cell signals during development. Salivary glands from Chironomus larvae, at various stages of development, were injected with a mixture of three different fluorophore-tagged molecules and the molecules' transit velocities through intercellular junctions were determined. The junctional transit velocities decreased markedly and selectively as development proceeded through the fourth (last) instar period. The larger the molecule, the larger were the velocity retardations. In many instances, at a certain developmental stage, junctional transit was blocked for the largest molecule of a given trio (e.g. 1440 daltons), while transit of the smaller molecule (e.g. 330 daltons) continued. This selective permeability reduction could be mimicked by injecting the larvae with 0.2µg of the developmental hormone ecdysterone. (Supported by NINCDS 5T32NS07044 and NIH CA14464).

W-AM-Po41 EXTRACELLULAR EGTA BY ITSELF DEPRESSES K^+ CURRENT IN LIMULUS VENTRAL PHOTORECEPTORS.
G. L. Fain*, M. C. Swan*, and J. E. Lisman+ (Intr. by J. Horwitz), UCLA* (Los Angeles) and Brandeis Univ.+ (Waltham, Mass.).

Depolarization of Limulus ventral photoreceptors in the dark from a holding potential of -70 mV evokes at least two components of outward current: one which is rapidly inactivated and preferentially blocked by external 4-aminopyridine, and another which is maintained even for 2 sec voltage steps and reduced preferentially by internal TEA. This latter, maintained current has been shown to be carried principally by K^+ (Pepose & Lisman, J. Gen. Physiol. 71, 101). 1 mM EGTA, added with $CaCl_2$ to ASW so that the free $[Ca^{2+}]$ was unchanged (at 10 mM), produced a marked reduction in the maintained outward current but had little effect on other outward or inward currents. Recovery was often incomplete and slow (10 min to 1 hr). The effect of EGTA did not depend upon the presence of an inward Ca^{2+} current, since suppression of outward current could still be observed after all the inward current had been blocked, in a solution containing 10 mM Ni^{2+} and zero (no added) Ca^{2+}. EGTA apparently acts on the external surface of the membrane, rather than by leakage into the cell. EGTA injected directly into the cytoplasm to produce a final estimated concentration of 30 mM resulted in a pronounced slowing of the waveform of light-induced currents, characteristic of large intracellular concentrations of EGTA (Lisman & Brown, J. Gen. Physiol. 66, 489), but reduced maintained outward currents in darkness by less than 10%. In contrast external EGTA, which markedly reduced outward currents, had little or no effect on light responses.

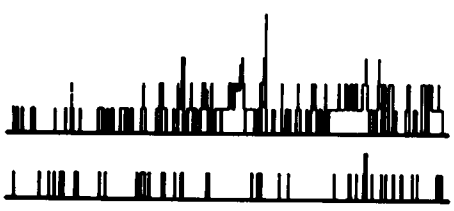
W-AM-Po42 EVIDENCE THAT FIVE CALCIUM IONS ACT COOPERATIVELY TO PROMOTE EVOKED ACETYLCHOLINE RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. R.S. Manalis* (Intro. by E. C. Foulkes), Dept. Physiol., UN. Cincinnati, Cincinnati, Ohio 45267.

Experiments were performed on frog (Rana pipiens) sciatic nerve/sartorius muscle preparations in order to re-investigate the relationship between extracellular calcium and evoked release of transmitter. Preparations were mounted in a suitable chamber; conventional intracellular microelectrode techniques were used. The Ringer solution contained (in mM): 111 NaCl;2.5 KCl; 2.9 $MgCl_2$; 4.0 TRIS maleate; and .20-.45 $CaCl_2$. The temperature was maintained at 15° C; the pH was 6.9-7.1. The average amplitude of the endplate potential (epp) was taken as a measure of evoked transmitter release. The nerve was stimulated only during recordings at 2 Hz. The slope of the relationship log $[Ca]_o$ versus log epp was found to be 4.11±.09 ($\bar{x}$±s.e) in 21 experiments. Linear regression analysis of data relating $[Ca]_o^{-1}$ to epp $^{-1/4}$ resulted in negative y-intercepts in 14 out of 21 experiments. Some plots of such data, in fact, revealed a nonlinearity which was present as an upward concavity at low $[Ca]_o$. The linearity of the reciprocal plots improved (and the y-intercepts were all positive) when fifth, instead of fourth, roots of the epp's were used. The model of Dodge & Rahamimoff(1967) was used to produce computer simulations of experimental results. The data obtained in these experiments are more consistent with the cooperativity coefficient(n)being equal to five rather than to four and are in agreement with the theoretical predictions made by Werman (Comp. gen. Pharmac., 2: 129, '71). Furthermore, it is concluded that an accurate measure of cooperativity can be obtained by finding the value of n which corresponds to the best straight line in a Lineweaver-Burk plot.

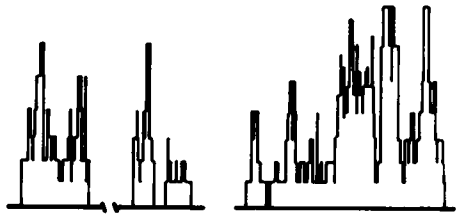
(Supported by NIH Grant ES00649.)

W-AM-Po43 MONTE CARLO SIMULATIONS OF SINGLE MODULAR GATING CHANNEL KINETICS. Gilbert Baumann and George S. Easton* (Intr. by Melvyn Lieberman), Department of Physiology, Duke University Medical Center, Durham, NC 27710.

Monte Carlo simulations of the microkinetics of a modular channel consisting of dipolar, amphiphilic subunit molecules that gate via aggregation predict two kinds of gating behavior. A channel can show (a) continuous single-step current fluctuations, or (b) multi-step current fluctuations that occur in bursts. At the macrokinetic level the set of parameters for (a) produces Hodgkin-Huxley-type kinetics, and for (b) alamethicin-like kinetics. A modular channel with the open-close gating behavior of (a) has the potential for high ion specificity. (Supported by NSF grant PCM78-02802).



(a) -125mV (above), -145mV (below)



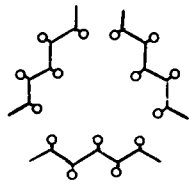
(b) -115mV (left), -114mV (right)

W-AM-Po44 NOVEL KINETIC FEATURES IN THE SODIUM CONDUCTANCE SYSTEM OF THE SQUID AXON. Gilbert Baumann and Gerry S. Oxford, Department of Physiology, Duke University Medical Center, Durham, NC 27710 and Department of Physiology, University of North Carolina, Chapel Hill,NC 27514.

The hypothesis of a modular gating channel made from amphiphilic, dipolar subunit molecules that opens and closes via step-by-step assembly and decay predicts in response to certain pulse sequences unusual voltage-clamp kinetic behavior that deviates from the kinetics given by the Hodgkin-Huxley formalism, a Hill-Chen-type aggregation model, or a sequential scheme with a terminal inactivation step. The guiding principle in the search for discriminating behavior was the recognition that a kinetic system reveals more characteristic properties when it is perturbed in a state removed from steady-state. The unusual kinetic features were experimentally revealed in the sodium conductance system of the voltage-clamped, perfused squid axon submitted to the same pulse sequences which discriminate among the above mentioned models. A molecular interpretation of the data in the context of the modular channel concept is given. The experimental results suggest that the modular channel concept is a plausible alternative to explain certain features of sodium channel gating behavior in excitable membranes. (Supported by NSF grants PCM78-02802 and BNS77-14702).

W-AM-Po45 ON THE STRUCTURES OF SCORPION AND SEA ANEMONE TOXINS AND THE SODIUM CHANNEL. H. R. Guy, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Scorpion and sea anemone toxins are relatively small proteins that bind to action potential Na^+ channels in a manner that alters either the activation or inactivation kinetics of the channel. A Chou and Fasmen analysis to the structure of scorpion toxins is consistent with their forming a triangle-shaped molecule that has parallel beta sheet conformations on each side, beta turns in its corners, and a hole through its center. If this conformation is correct, the scorpion toxins should bind to the top of a triangle-shaped membrane channel whose sides are also in the beta sheet conformation (see figure of Tri beta channel). One can use the Tri beta backbone structure to design a channel that is consistent with the following phenomena associated with Na^+ channels: (a) binding of scorpion and sea anemone toxins, (b) permeability to organic cations, (c) blockade by tetrodotoxin and saxitoxin, (d) blockade by local anesthetics, pancuronium, strychnine, diphenylhydantoin, and barbiturates, (e) effects of batrachotoxin, veratridine, aconitine, and grayanotoxin, and (f) m^3 activation kinetics. The Tri beta channel model can be tested by synthesizing a polypeptide which, when incorporated into a lipid bilayer, should form a triangular beta helix with the appropriate dimensions and side chains.



Tri beta channel

W-AM-Po46 DEVELOPMENTAL CHANGES IN ELECTRICAL PACEMAKER PROPERTIES OF AGGREGATES OF CHICK EMBRYO VENTRICULAR CELLS OBSERVED UNDER CURRENT AND VOLTAGE CLAMP. A. Shrier, J.R. Clay and R.L. DeHaan, Department of Anatomy, Emory University, Atlanta, GA 30322

We have observed differences in spontaneous activity of small spheroidal aggregates of ventricular cells prepared from hearts of 7, 12, and 17 day old chick embryos. In low K^+ tissue culture medium (K_e^+ = 1.3mM) 7 day aggregates beat spontaneously and rhythmically, 12 day preparations either beat spontaneously or are quiescent, and 17 day clusters are completely quiescent. We have analyzed the pacemaker currents in all three preparations using the two microelectrode current- and voltage-clamp techniques. TTX ($3x10^{-6}$M) was applied to block the inward current associated with the fast upstroke of the action potential. Under these conditions the 7 day preparation rests near -50mV and exhibits a time dependent conductance between -90 and -60mV (Clay, Shrier and DeHaan, these abstracts). The 12 day preparation has two resting states, one near -90mV and one near -60mV. Moreover, this preparation exhibits large (30mV) non-linear oscillations between the two rest levels when steady current is applied. The steady state IV curve is N shaped with zero net current points near -90, -75, and -60mV. No time dependence was observed in the current responses to voltage clamp steps from holding potentials between -90 and -70mV. Time dependent currents were observed from more positive holding potentials. The 17 day preparation also exhibits two rest states, near -90 and -70mV and a corresponding N shaped steady state IV curve. However, this preparation lacks voltage oscillations and has a smaller time dependent current than that observed in the 12 day preparation. (Supported by the Canadian Heart Foundation and NIH HL16567, 05346.)

W-AM-Po47 STEROL DEPENDENCE OF THE ELECTRICAL PROPERTIES OF LIPID BILAYERS IN THE PRESENCE OF PENTACHLOROPHENOL. A.D. Pickar and J. Hobbs. Portland State University, Portland, Oregon 97207.

The frequency dependence of membrane admittance has been determined for a series of lecithin/sterol/decane bilayers in the presence of an aqueous environment containing pentachlorophenol (PCP). The results are tentatively interpreted in terms of a three-element circuit consisting of resistor-capacitor pairs representing surface and interior properties of the membrane. Membranes formed with β-hydroxysterols having a flat ring structure and an intact side chain (cholestanol, cholesterol, and 7-dehydrocholesterol) exhibit an initial rise followed by a sharp drop in surface conductance as the fraction of sterol is increased. Interior conductances rise monotonically with sterol concentration. Other sterols (stigmasterol, coprostanol, and epicholestanol) exhibit much weaker effects. The results are interpreted in terms of sterol induced dipolar field changes which act differentially on proton transport at the surface and on negatively charged PCP-dimer transport across the interior. Sterol induced viscosity changes also affect transport in the hydrocarbon interior. Dipolar field changes are presumably due both to an alteration in the intrinsic field of the lecithin-water interface and to the superposition on this of sterol dipole fields. The differences observed among the several β-hydroxysterols suggest that each type lies at a different depth within the membrane in the sequence: cholestanol, cholesterol, 7-dehydrocholesterol, stigmasterol (deepest). After correction for electrostatic effects associated with sterol-induced membrane thinning the interior conductance data support the view that in lecithin membranes cholesterol produces viscosity and dipolar field changes which largely cancel in the hydrocarbon region.

W-AM-Po48 ELECTRIC CURRENT FLOW IN FROG ATRIAL TRABECULAE AND THE SMALL SIGNAL IMPEDANCE MEASURED WITH THE DOUBLE SUCROSE GAP. R. McKown, Dept. of Physiology, Emory University, Atlanta, GA 30322.

Electric current flow in the multicellular trabecular tissue of frog atria is modeled by treating the membrane network inside an annulus of tissue as a source of extracellular current and a simultaneous sink of intracellular current. The impedance measured from resting trabeculae are shown to be consistent with the extracellular radial cable version of this dual space "Poisson" model and inconsistent with lumped parameter models. If the membrane specific capacitance is assumed to be 1 microfarad/cm^2, the 10 Hz corner frequency of the impedance amplitude provides a 16 Kohm-cm^2 estimate of the specific resistance of the resting atrial cell membrane. Small signal impedance measurements at different mean holding voltages provide an impedance surface containing a low frequency (~1 Hz) resonance feature. The resonance is maximized at holding voltages 40 to 50 mV above rest and is qualitatively consistent with the 1962 Nobel Model for the nonlinear characteristics of potassium currents through the heart cell membrane.

W-AM-Po49 ELECTRICAL IMPEDANCE OF THE PERINEURIUM OF THE FROG SCIATIC NERVE. A. Weerasuriya,* R. Spangler, S. Rapoport, and R. Taylor (Intr. by G. Ehrenstein), NINCDS, NIH, Bethesda, Md. 20014; SUNY, Buffalo, N.Y. 14214; NIA, NIH, Baltimore, Md. 21224.

An experimental arrangement consisting of four electrodes has been developed to measure the impedance in vitro of the cylindrical perineurium at frequencies from 1 Hz to 0.1 MHz. The D.C. resistance of the perineurium is 431±27Ω cm^2 (S.E.M., n=31). Impedance measurements of the normal perineurium reveal two dispersions with center frequencies at about 5 KHz and 20 Hz. Perineurial impedance was also measured in (1) Ringers in which 90% of the NaCl was replaced by an equiosmolal amount of sucrose and (2) in normal Ringer before and after the tissue had been immersed for 10 minutes in hypertonic Ringer containing either an additional one or two molal of NaCl. Whereas exposure to hypertonic Ringer decreases the D.C. resistance of the perineurium, the low conducting medium increases it. The above data were fit to the four possible, four-element (two resistors and two capacitors) equivalent circuits with two time constants. Increasing the degrees of freedom in the models with a third resistor did not significantly enhance the goodness of the fit but the uniqueness of the fit was decreased. Analysis of the four-element models reveals that the capacitance of the higher frequency dispersion is unchanged by the experimental manipulations and is approximately 0.1μF/cm^2. If this capacitance is due to perineurial cell membranes, then it suggests the presence of about five layers of cells in the perineurium. The capacitance of the low frequency dispersion can be altered by the experimental manipulations and this capacitance is thought to represent properties of the extracellular matrix of the perineurium.

W-AM-Po50 MOLECULAR MOTIONS OF MEMBRANE AGGREGATES: A SATURATION TRANSFER EPR STUDY. W. A. Meena and L. W.-M. Fung, Department of Chemistry, Wayne State University, Detroit, Michigan 48202

Saturation transfer electron paramagnetic resonance (ST EPR) spectroscopy has been used to study membrane proteins of human erythrocytes. A spin label, N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) maleimide (Mal 6), is covalently attached to the sulfhydryl groups of spectrin and of proteins in intact membranes. Conventional spin-label EPR studies indicate that these proteins have predominately strongly immobilized motion in membranes. However ST EPR, a method with extended sensitivity to slow motions, allows us to monitor these slow molecular motions quantitatively and furthermore to follow the motion during the process of membrane aggregation. the spectra generally show slow anisotropic motion in the time range of 10E-7 to 10E-3 seconds. The effects of pH and calcium concentration on the motion of spectrin and proteins in membrane ghosts have been studied. We have also explored the effects of the spin label Mal 6, an NEM analogue, on aggregation of spectrin and of membranes. The aggregates collected by high-speed centrifugation seem to have slower motion than those collected by gravitational sedimentation. These results may be related to the different forms of aggregates observed by negative staining electron microscopy.

(Supported by NIH grant HL 22432, Michigan Heart Association and Research Cooperation.)

W-AM-Po51 THE EFFECT OF INSULIN ON THE MEMBRANE POTENTIAL OF HEART CELL AGGREGATES.
R.C. Lantz*, L.J.Elsas* and R.L.DeHaan, Dept. of Anatomy and Division of Medical Genetics, Dept. of Pediatrics, Emory University, Atlanta, GA 30322

Spheroidal aggregates were formed from trypsin-dissociated 14 day embryonic chick hearts by 48 hrs. of rotation on a gyratory shaker. Intracellularly recorded resting membrane potentials of aggregates bathed in 2.5 mM K^+ balanced salt solution, ranged from -65 to -71 mV. After achieving a stable membrane potential, addition of 2 µg/ml of sodium bovine insulin caused a slow hyperpolarization which reached a peak of 10 to 12 mV after 5 minutes. The possibility that the insulin-induced hyperpolarization (IIH) may be caused by enhanced Na-K pump activity was negated by the finding that the IIH was still present after addition of 10^{-6} M ouabain. We conclude that the IIH more probably results from a ouabain-insensitive pump or from a direct effect of insulin on specific ion permeability of the membrane. This research was supported by grants HL16567 and 1-T32-AM07298-01.

W-AM-Po52 A COMPARATIVE STUDY OF IMPEDANCE AND PHASE OF NERVE AND HEART CELL MEMBRANES.
D.E. Clapham and L.J. DeFelice, Department of Anatomy, Emory Univ., Atlanta, GA 30322

The small signal impedance of the membranes of squid axon, frog node of Ranvier, Purkinje heart fibers and ventricular tissue have been determined by linearizing the respective voltage clamp equations. Both modulus ($|Z|$) and phase of the impedance have been calculated in each case for the entire voltage and frequency ranges of physiological interest. Impedance or phase is plotted against voltage and frequency axes to yield a "3-dimensional" view of these surfaces. Resonant peaks in $|Z|$ appear in all surfaces with corresponding phase changes. In squid axon two resonant peaks in $|Z|$ appear,at-55 mV, 93 hz and at -46 mV, 166 hz. The node of Ranvier exhibits one broad resonance with maximum near -65 mV, 250 hz. The Purkinje fiber model and ventricular models exhibit 4 and 3 resonant peaks respectively, all below 1 hz. Repetitive activity of these membranes roughly corresponds to the frequency for which the resonances are predicted. In all cases resonant peaks appear at approximately threshold voltages. The effect of removal or blockade of various ionic systems of the membranes has also been computed. Although use of linearized impedance surfaces is not justified for the large signal nonlinear range of action potentials, such calculations can accurately predict small signal variations (Mauro, *et al.*, 1970)[1].

A more limited range of the experimental small signal impedance surface of the voltage-clamped heart cell aggregate is compared to the theoretical calculations for heart tissue.

[1]Mauro, A., Conti, F., Dodge, F., and R. Schor. 1970. J. Gen. Physiol. 55: 497-523.

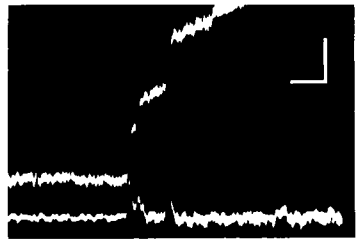
W-AM-Po53 VOLTAGE CLAMP ANALYSIS OF PACEMAKER CURRENTS IN EMBRYONIC HEART CELL AGGREGATES.
J.R.Clay, A.Shrier and R.L.DeHaan, Dept. of Anatomy, Emory University, Atlanta,GA 30322

We have recorded membrane currents from spheroidal aggregates 200 µm in diameter using the two microelectrode voltage clamp technique. Aggregates were prepared from 7 day old chick embryonic ventricular cells and maintained in medium containing 1.3mMK^+. TTX (3×10^{-6}M) was added to block the fast inward current which is normally activated between -50 and -60mV. The zero net-current point of the steady state IV curve lies between -48 and -58mV, close to rest potential (E_r). Between E_r and -90mV net membrane current is inward. Its amplitude at -90mV is 10-15nA. Rectangular voltage clamp steps in this potential range reveal a conductance change which is time- and voltage-dependent. This conductance is activated between -60mV and -90mV, as revealed by the tail current amplitudes which describe a sigmoidal function over these potentials. The tail and the "on" currents are both single exponential functions of time. The "on" current time constant is a function of the clamp step potential, whereas the tail current time constant depends only on the holding potential. The voltage dependence of all the time constant data can be described by $(\alpha+\beta)^{-1}$, and the tail current amplitudes can be described by $\beta/(\alpha+\beta)$, where $\alpha = 1.6\ (V+52)/1-\exp(-.2(V+52))$ sec^{-1}, $\beta = .13\ \exp(-.067(V+52))$ sec^{-1}, and V is membrane potential. These results are consistent with a channel which is fully open at -90mV and fully closed at -60mV. This model channel, which is closed by depolarization in low-K medium, is consistent with that derived from membrane noise and impedance measurements of Clay, DeFelice and DeHaan (Biophys. J. 21: 166a, 1978) in similar preparations in medium containing 4.5mMK^+.

(JRC is an NIH Research Fellow. AS is a Fellow of the Canadian Heart Foundation. This research was also supported by NIH HL16567.

W-AM-Po54 MEMBRANE NOISE AND SUCCESSFUL COLLISION RATES STUDIED DURING MULTIPLE SPERM-EGG INTERACTIONS. L.J. DeFelice and B. Dale.* Anatomy Department, Emory University, Atlanta, GA, 30322 and Zoological Station, Naples, Italy, 80121.

The successful collision of a single spermatozoon with an egg is accompanied by a step depolarization, an increase in voltage noise and a decrease in resistance of the egg plasma membrane (Nature 275, 217 (1978)). Recently we have studied multiple sperm-egg interactions using two microelectrodes. Successful collisons appear to occur randomly. A monospermic response consists of a 1-2mV step from a resting potential of about -15mV. The step lasts approximately 13 sec at 20°C. If other successful collisions occur during this period (indicated by additional steps) the egg becomes polyspermic. There is a further noise increase and resistance decrease at each step. No evidence for an electrically mediated fast block to polyspermy was found. The average rate of successful collisions increases monotonically with sperm density. Our collision rates agree with earlier results (Rothschild and Swann, J. Exp. Biol. 29, 469 (1952)) for sperm densities up to 10^6/ml; for higher sperm densities, our method giving higher collision rates. The Figure shows an intercellular recording from a Psammechinus egg inseminated with 10^7 sperm/ml. Rest potential -12mV. Temp: 22°C. The top trace is dc-coupled (Scale: 1mV). Three distinct steps are seen. The lower trace is ac-coupled (Scale :0.4mV). Note noise increase for each step. Time scale: 1 sec.



W-AM-Po55 PROPERTIES OF A FAST FLUORESCENT DYE FOR MEMBRANE POTENTIALS IN HUMAN RED BLOOD CELLS. Jeffrey C. Freedman and Joseph F. Hoffman, Department of Physiology, Yale University School of Medicine, New Haven, CT 06510.

For some experiments involving the membrane potential of human red blood cells, an optical probe which responds faster than the permeant cyanine dye, diS-$C_3(5)$, would be desirable. For this purpose an asymmetric pentamethin oxonol dye with 1,3-dibutylbarbituric acid-(5) and 1, (p-sulfophenyl) 3-methyl, 5-pyrazolone (4) groups, denoted WW 781, was tested. This dye was chosen since it gives large fluorescence (F) signals for optical monitoring of action potentials in squid axons (L.B. Cohen, et al., Biol. Bull. 153: 419, 1977). Upon adding normal human red blood cells to isotonic medium containing dye, F increased to 18 times that of dye in buffer alone. Using a 600 ± 10 mμ narrow bandpass excitation filter and an emission cut-on filter at 645 mμ, a change in F was induced by VALinomycin (1 μM final conc.) at low external K (K_o). This change was maximal at 1.2% hematocrit and 0.24 μg/ml dye. At 1 mM K_o, F decreased by 7.3 ± 0.6%, and at 150 mM K_o, F increased by 1.3 ± 0.6% (SD, n=3), with a nonlinear curve of $\%\Delta F$ vs log K_o between 1 and 150 mM. The null point, or K_o at which F does not change with VAL, is similar to that obtained with diS-$C_3(5)$. The signal to noise ratios are such that diS-$C_3(5)$ detects 1 mV signals while WW 781 has a detection limit of 10 mV. The half time for the response of WW 781 is less than or equal to the half time of mixing (0.5 sec) for our sample chamber, faster than diS-$C_3(5)$ by at least a factor of 10. Split beam absorption spectra of cells + dye + VAL vs cells + dye showed no absorption shifts at a detection limit of .005 OD. (We thank L.B. Cohen and A.S. Waggoner for dye samples.) (Supported by USPHS grants HL-09906 and AM-17433.).

W-AM-Po56 CHARGE SHIFT PROBES OF MEMBRANE POTENTIAL. CHARACTERIZATION OF AMINOSTYRYL-PYRIDINIUM SYSTEMS IN LIPID VESICLES AND HEMISPHERICAL BILAYERS. L. M. Loew, L. Simpson*, A. Hassner*, and H. Parkins*, Department of Chemistry, SUNY at Binghamton, Binghamton, New York 13901.

Theoretical calculations on the 4-[p-aminostyryl]-1-pyridinium chromophore predicted a large shift in charge upon excitation (L. M. Loew, G. W. Bonneville and J. Surow, Biochemistry,17, 4065, 1978). Several derivatives of this chromophore have been prepared with substituents designed to assure strong binding and to preclude flip-flop in a membrane; also, the substituents are placed so as to favor orientation of the chromophore long axis perpendicular to the membrane surface. These systems are, thus, optimized for sensitivity to membrane potential by a direct electrochromic mechanism. Absorbance, fluorescence and polarization spectra of the probes in lipid vesicles or macroscopic hemispherical bilayers are used to characterize these systems with respect to binding, orientation, degree of aggregation, and solvatochromism. The response of the probes to potential pulses across the hemispherical bilayers is determined as a function of bulk probe concentration, pulse width, pulse amplitude, polarization of the incident light, and wavelength. The results of these experiments are inconsistent with aggregation or redistribution mechanism which have been postulated for several of the cyanine and merocyanine extrinsic optical potential probes. Further, the similarity of the excitation action spectra of these charge shift probes to the first derivatives of their absorbance spectra, provides positive evidence for an electrochromic mechanism. (Supported by NIH grant GM-25190)

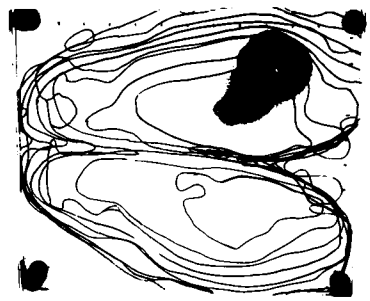
W-AM-Po57 AN ANALYSIS OF VOLTAGE FLUCTUATIONS AT THE FROG MOTOR ENDPLATE IN RESPONSE TO A COVALENTLY-BONDING ACTIVATOR. Robert N. Cox*, Arthur Karlin*, and P.W. Brandt, Depts. of Neurology, Biochemistry, and Anatomy, Columbia Univ., N.Y., N.Y. 10032.

The cholinergic receptor of the frog sartorius motor endplate was treated with the specific disulfide bond reducing agent dithiothreitol (DTT) and subsequently exposed to a covalently-bonding compound (the p-nitrophenyl ester of p-carboxyphenyltrimethylammonium, NPTMB) known from previous work to activate the DTT-reduced eel electroplaque receptor. NPTMB causes maximum depolarizations of about 35 mV when applied to the DTT-treated sartorius motor endplate. It is ineffective on postjunctional membrane prior to disulfide bond reduction and on extrajunctional regions, treated or untreated. High concentrations of a competitive antagonist such as d-tubocurarine will prevent covalent reaction between NPTMB and the altered sartorius receptor and will cause a repolarization of the membrane when applied to the already-depolarized preparation. These results are in good agreement with those obtained on the eel electroplaque. It is concluded that NPTMB bridges the active site of the reduced receptor, interacting between a sulfhydryl group, to which it is covalently bound, and the receptor anionic locus, with which it forms a reversible ionic bond. Molecular properties of the covalently-activated sartorius receptor have been investigated with the technique of fluctuation analysis. The depolarization induced by NPTMB is accompanied by a small increase in voltage fluctuations. The amplitude of the elementary voltage event produced by NPTMB is about .04μV, almost an order of magnitude below the acetylcholine shot effect amplitude in the control preparation, and about one-fourth the acetylcholine shot amplitude after disulfide bond reduction. Spectral density plots of NPTMB noise can be analyzed in terms of two relaxation rates which bracket the single rate observed in response to acetylcholine. Supported by NIH 5R01-NS05910-11.

W-AM-Po58 THREE DIMENSIONAL VISUALIZATION OF A REDOX WAVE OF SPREADING DEPRESSION IN A GERBIL BRAIN. J.C. Haselgrove, A. Mayevsky*, B. Chance, and E. Joyce*. Johnson Research Foundation, Univ. Penn., Philadelphia, Pa, 19104

We have used fluorescence signals from NADH and from flavoproteins to map the 3 d distribution of metabolic states in a gerbil brain during spreading depression. The brains of anaesthetized animals were "funnel frozen" with liquid nitrogen at different periods (0 to 70 secs) after the initiation of the spreading depression. On each brain, a series of 2d scans of NADH and flavoprotein fluorescence were made at different levels through the brain by successively grinding away the surface and scanning the new surface (see Quisdorff, B.T., Chance, B. in "Oxygen and Physiol. function," Ed. Jobsis, Prof. Inf. Library '76).

Each brain was scanned to a depth of 1.6mm. The wave of spreading depression appears as a region of reduction about 2mm wide which spreads in the plane of the cortex at 2.3 mm/minute which is similar to the electrical recordings of Leao (J. Neurophysiol. 10:404 (.47)): the wave appears to propagate more rapidly in the white matter than in the grey.



This work was supported by grants. USPHS NS10939, AM-19525, HL-18708

W-AM-Po59 TWO DIMENSIONAL FLYING SPOT FLUORIMETRY OF OXONOL V IN THE GERBIL CEREBRAL CORTEX J. C. Smith, C. L. Bashford, B. Chance, B. R. Silberstein*, J. C. Haselgrove, and E. Joyce* Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 USA

The potential sensitive probe oxonol V, applied epidurally to the exposed gerbil cerebral cortex, responds to anoxia induced by N_2 inhalation by a decrease of dye fluorescence relative to the normoxic state as indicated by the histogram displays of flying spot fluorescence (Chance, et al, Am. J. Physiol., in press). The half time for the response is 10 - 15 seconds and the fluorescence histogram reverts to the normoxic level within a five minute recovery period. Using 590 nm excitation from a dye laser, conditions where intrinsic reflectance changes are minimal, the oxonol fluorescence may decrease by as much as 50 percent during anoxia according to the change in histogram position. Much smaller changes are induced by carotid artery occlusion, possibly because of compensating circulation from the vertebral arteries. Spreading depression, initiated by 0.5 M KCl, causes oscillation of the oxonol fluorescence and reflectance histogram peak positions for at least 20 minutes as the dye responds to successive waves of depolarization. Excised rat brains accumulate 50 μM oxonol V with a half time of roughly 20 minutes; freezing and scanning indicate that the depth of dye penetration was about 100 microns. The possible use of oxonol V as a measure of tissue distributions of membrane potential in frozen samples is indicated by the observation that the dye response to membrane energization in submitochondrial particles is preserved when the preparation is frozen at 77°K. This work was supported by U. S. P.H.S. grants NINCDS NS 10939, HL 18708 and HL 17826.

W-AM-Po60 EFFECT OF Ca^{+2}, Mg^{+2}, AND OTHER AGENTS UPON THE SURFACE CHARGE AND AGGREGATION OF GUINEA PIG CEREBRAL CORTEX SYNAPTIC VESICLES. D. P. Siegel and B. R. Ware, Chemical Laboratories, Harvard University, Cambridge, MA 02138

Secretion of neurotransmitter in the central nervous system is generally believed to involve the fusion of secretory vesicles within the nerve terminal with the pre-synaptic membrane, followed by discharge of the vesicle contents into the synaptic cleft. An influx of Ca^{+2} into the terminal is the stimulus for this process; Mg^{+2} will not substitute. The specific role of Ca^{+2} vs. Mg^{+2} remains unclear. Using the technique of electrophoretic light scattering (ELS), in which the Doppler shift of laser light scattered from particles moving in an electric field is analyzed to derive the electrophoretic velocities and average size of the particles, we have demonstrated that Mg^{+2} and Ca^{+2} are equally effective in reducing the surface charge of the vesicles. It is therefore unlikely that the Ca^{+2}-specific process is reduction of the electrostatic repulsion between the like-charged secretory vesicles and pre-synaptic membrane. Results concerning the effects of these and other agents upon the surface charge and aggregation of synaptic vesicles will be presented.

W-AM-Po61 ACETYLCHOLINE SYNTHESIS IN THE GIANT NERVE FIBER OF THE SQUID. R. Heumann* and J. Villegas, Max-Planck-Institut fur Biochemie, D-8033 Martinsried bei Munchen, BRD, and Centro de Biofísica y Bioquímica, IVIC, Apartado 1827, Caracas 101, Venezuela.

The present communication deals with the detection and measurement of choline acetyltransferase enzymatic activity (EC 2.3.1.6, CAT) in the isolated giant nerve fiber of the tropical squid *Sepioteuthis sepioidea*. Pooled samples of intact giant nerve fibers (6-29 per sample), extruded axoplasm (40-43), and axoplasm free giant nerve fiber sheaths (24-25), were analyzed by means of a specific radiochemical test (Hamprecht, B & Amano, T., Anal. Biochem. 57: 162-172, 1974) which discriminates between choline acetyltransferase and carnitine acetyltransferase activities. The total protein content measured in each different sample (in mg), was: intact fibers, 0.6, 2.7, 5.0; extruded axoplasm, 3.4, 3.5; sheaths, 1.3, 1.0. The values found for CAT specific activity in these groups of nerve fibers (in $pmole.min^{-1}.mg^{-1}$), were: intact fibers, 160, 210, 50; axoplasm, 20, 16; sheaths, 70, 180. In addition, the samples of intact fiber and extruded axoplasm contained carnitine acetyltransferase activity, whereas it could not be detected in the axoplasm-free sheaths. These experimental findings show the presence of CAT in the giant nerve fiber of *S. sepioidea*, and indicate that the activity of this enzyme is about 3-9 times higher in the periaxonal sheaths than in the axoplasm. Thus, it is most likely that in these nerve fibers the Schwann cells, which form about 84 percent of the total cellular volume in the sheaths, are able to synthesize acetylcholine.

W-AM-Po62 PASSIVE CABLE PROPERTIES OF HIPPOCAMPAL NEURONS. D. Johnston, Baylor College of Medicine, Houston, Tx. 77030.

The cable properties of CA3 neurons in the hippocampus were determined using both current and voltage clamp techniques in order to evaluate the feasibility of studying voltage dependent ionic conductances in these neurons. 400-500 μm thick slices of guinea pig hippocampus were maintained using standard techniques. Current and voltage clamping were performed with a single microelectrode clamping system. Data were obtained in the following manner: (1) measuring the multiple exponentials in the voltage decay following a current clamp pulse, (2) measuring the multiple exponentials in the current decay following a voltage clamp pulse. Each method yields two sets of time constants, but each set can be used to independently determine neuronal cable properties, based on the theory developed by Rall. The average electrotonic length of soma and dendrites from 15 neurons was 0.9 space constants. The dendritic to soma conductance ratio was always less than 5 with an average for 14 neurons of 1.8. Cable properties calculated from the two sets of data compared favorably, although the voltage clamp measurements always produced shorter electrotonic lengths and smaller G_d/G_s ratios. The results suggest that a reasonably good space clamp can be maintained with certain time resolution limitations. The data also suggest that the specific membrane resistivity in these neurons is very large. Supported by NIH Grant NS11535.

W-AM-Po63 AMINO GROUP REAGENTS AFFECT INACTIVATION IN SQUID AXON. Douglas C. Eaton and Malcolm S. Brodwick, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The reagents 4-acetamido-4-isothiocyano-stilbene-2,2'disulfonic acid (SITS), trinitrobenzene-sulfonic acid (TNBS) and isethionyl acetamidate hydrochloride (IAH) react in a highly specific fashion with the - amino group of lysine residues and the N-terminal amino groups of proteins. When applied internally to perfused squid axon each of these reagents removes Na^+ inactivation in a manner that is similar to various proteases. Both SITS and TNBS when applied externally also remove inactivation suggesting that some simple models of inactivation may be incomplete. However, when the inactivation curves after SITS treatment are examined, one observes that removal of inactivation is more complete at large depolarized potentials or for depolarized test pulses. This voltage dependence of the removal of inactivation is less marked for TNBS and not substantially present after treatment with IAH. The correlation is interesting since on reaction with an amino group, SITS replaces the positively charged amino group with a doubly negatively charged group, TNBS with a singly negatively charged group and IAH leaves the charge unchanged. Additionally, when applied internally, SITS, to a greater extent, and TNBS, to a lesser extent, produce shifts of the current voltage relationship in a direction consistent with adding negative charge to the inner membrane surface. The effect of SITS and TNBS on the K^+ current is a slowing of kinetics by as much, in some cases, as two orders of magnitude.

W-AM-Po64 EXCITABLE MEMBRANE CURRENTS PROCEED AT EXPONENTIALLY CHANGING RATES. Dexter M. Easton, Dept. of Biological Science, The Florida State University, Tallahassee, Florida, 32306

The power function required to describe the conductance change in the Hodgkin-Huxley axon reflects the inadequacy of standard kinetics in dealing with rate processes. A more realistic model may be developed from the assumption that membrane current changes exponentially with time and that the rate coefficient of that change decreases exponentially with time. Thus a meaningful rate constant replaces an arbitrary power. The relation of current to voltage step follows from the assumption that r, the specific rate of growth, increases exponentially with voltage, but the voltage rate coefficient of r decreases exponentially with voltage. Thus the relation of current to voltage is not found empirically through changing rate constants, but follows from initial assumptions about the behavior of rate coefficients. In standard kinetics, treating "ideal" situations, the proportional change of y with respect to x is assumed to remain constant during a process, and corrections are made to account for deviations from reality. Expo-exponential kinetics assumes that in the real world the rate coefficient of a process changes (exponentially) with the independent variable. With that assumption, membrane current for a specific ion species during any voltage step may be modelled with a pair of time rate constants, a voltage rate constant, and specification of initial conditions. (Aided by FSU Computer Center and Psychobiology Program).

W-AM-Po65 THE NATURE OF THE MEMBRANE POTENTIAL OF SCHWANN CELLS OF CRAYFISH VENTRAL NERVE CORD. E. M. Lieberman, J. Villegas and G.M. Villegas*, Dept. of Physiology, East Carolina U. Sch. Med., Greenville, N.C. 27834 and Centro de Biofisica y Bioquimica, (I.V.I.C.), Apartado 1827, Caracas 101, Venezuela.

Electrophysiological, pharmacological and electron microscopic methods were used to characterize Schwann cells associated with the medial giant axon of the crayfish. In cross section, the axon is surrounded by 4 or 5 Schwann cells. The Schwann layer ranges from 0.2 to 1μ thick in the non-nuclear region to 3μ in the nuclear region of the cell. Membrane potentials of the giant axon and the Schwann cell were monitored before and during treatment with ouabain (10^{-3}M), carbachol (10^{-7}M) and acetycholine (10^{-9}-10^{-7}M). The membrane potential of 40 Schwann cells from 23 axons averaged -42±1mV (range -35 to -54mV). In paired experiments 10^{-3}M ouabain caused Schwann cells to hyperpolarize from the control level of -43±1mV (n=11) to -57±1mV (n=10). The effect was maximal within 2 min. The hyperpolarized level was stable for up to 18 minutes after which there was a rapid decline of the resting potential. While the Schwann cell was hyperpolarized in ouabain the axon was depolarized approx. 7mV and remained stable for the duration of the experiment. Both carbachol (10^{-7}M) and acetylcholine (10^{-7}M) caused the Schwann cell to hyperpolarize from the control level (-40mV) to 57±1mV (n=11 and n=7 respectively). The onset of the effect was rapid and reversible with 10-30 min. of washing. There was no significant effect of either ACh or carbachol on axon resting membrane potential, action potential or membrane resistance at the concentrations used in this study. The results show that physiologically and pharmacologically Schwann cells of crayfish nerve tissue are similar to Schwann cells of the tropical squid Sepioteuthis sepioidea. This study shows that it is possible to study crayfish axons and their surrounding Schwann cells, simultaneously. Supported, in part, by NSF BNS 77-28510.

W-AM-Po66 BLOCK OF SODIUM INACTIVATION IN NERVE BY POLYPHENOLS; ION ACCUMULATION IN THE SCHWANN CELL SPACE. P. Shrager and J.G. Starkus, Dept. of Physiology, University of Rochester Medical Center, Rochester, N.Y. 14642

Tannic acid is a polyphenol (MW 1701) composed of 4 to 5 digallic acid residues bound to a glucose backbone. When perfused internally in voltage-clamped crayfish giant axons, 10-30 μM tannic acid produces a complete loss of inactivation of sodium conductance. Effects are irreversible on removal of the compound from the perfusate after just a few minutes exposure. Two derivatives of tannic acid and a number of analogs have been tested for activity. 310 μM digallic acid perfused internally prevents Na inactivation in a manner similar to that of tannic acid. Gallic acid is much weaker, even at a ten-fold higher concentration. Pyrogallol, a triphenol that is rapidly oxidized to larger polymeric forms, blocks Na inactivation after several minutes at 0.5 mM internally. Phenol (5 mM) has only slight effects on Na currents, but slows K currents. Unlike the above compounds, effects of phenol are rapidly reversed on washing. Results with compounds tested thus far suggest that irreversible block of Na inactivation requires a compound with a minimum of two rings and multiple phenolic groups, and imply that crosslinking of at least two membrane sites may be involved.

After exposure to tannic acid, and reversal of the concentration gradient for Na^+, large, maintained, outward Na currents are elicited on depolarization. We have used these conditions to measure the accumulation of Na ions moving through Na channels into the Schwann cell space. Results of these experiments will be compared with those obtained for K ion accumulation in crayfish fibers.

Supported by NIH grant NS 10500 and RCDA 5 K04-NS00133.

W-AM-Po67 TEMPORAL CONTROL OF POTENTIAL IN A GIANT AXON VOLTAGE CLAMP. R. Levis, Dept. of Physiology, Rush University, Chicago, IL 60612

Several voltage clamp circuits have been analyzed by a combination of analytical and numerical techniques in order to optimize speed and compensation of resistance (R_S) in series with the membrane. The axon has been modeled by a lumped equivalent circuit. Numerical simulations have included HH nonlinear conductances, dynamic characteristics of the amplifiers (e.g., single or multiple pole roll-off, finite DC gain, input and stray capacities), and approximate equivalent circuits of the electrodes. Analytical expressions have dealt with more simplified situations but include characteristics of electrodes and important amplifiers. It is concluded that a fast, smooth settling clamp can be built which can in principle compensate for all R_S between the differential voltage electrodes. Traditionally, it has been found that as R_S compensation is increased, damping is not independent of the level of compensation; it becomes progressively more difficult to damp the system and instability eventually results. In the designs developed here, matching the frequency response of the voltage electrode/follower system to that of the I to V converter (used in R_S compensation) allows the damping and response time of the true transmembrane potential to be set independently of the amount of R_S compensation, as compensation approaches 100%. Although maximum clamp speed will depend on the degree to which such matching can be accomplished, perfect matching is not required if the voltage electrode system is faster than the I to V converter. Consequences of frequency response mismatch are considered. Existing amplifiers and electrodes should permit settling of the true transmembrane potential to within 1% of the command potential in about 1 μsec with nominal squid axon parameters. Rules for setting damping and response time are developed. Both simple modifications of traditional voltage clamp circuits and more exotic designs are considered.

W-AM-Po68 THE EFFECTS OF VERATRIDINE AND VERACEVINE ON THE CONDUCTED ACTION POTENTIAL OF THE MEDIAN GIANT AXON OF EARTHWORM. V. Verselis*, P. Brink and L. Barr, SUNY at Stony Brook and University of Illinois, (sponsored by R. Levine).

Veratridine has been shown to induce a slow Na inward current in frog node (Ulbricht, E. Phy. Bio. Chem. Exp. Phar., 1969) and to reduce the amplitude of the AP with concentrations in the μM range. The resting potential of the earthworm axon is slightly depolarized or unchanged in the presence of 1 μM veratridine but repetitive stimulation causes a prolonged depolarization with a rising phase time constant of 5 sec and a variable time constant of decline depending on the duration of stimulation (Td=50 sec-2 min). Decreasing extracellular Na showed the amplitude of the rising phase time constant. The stimulus dependent depolarization was dependent on the veratridine concentration suggesting voltage dependent binding of veratridine. In addition, 1 μM veratridine causes an increase in conduction velocity (20% increase) followed by a reduction in conduction velocity and finally conduction failure. With lower doses of veratridine (10 nM) only a velocity increase was observed (20%). Wash out of veratridine partially reduced the effect from 20 to 15% increase. The method used to monitor conduction velocity is given in Brink and Barr (J.G.P., 1977).

Veracevine (1 μM) causes no membrane depolarization upon stimulation but caused a velocity increase (30%). The possible mechanisms by which these drugs act on the action potential will be discussed.

W-AM-Po69 STOCHASTIC FLUCTUATIONS: THEIR ROLE IN THE CONTROL OF STATE IN NON-EQUILIBRIUM SYSTEMS. F. F. Offner, Northwestern University, Evanston, IL 60201.

On a molecular level, elements of a system will fluctuate between states because of stochastic conformational changes or binding of ligands. If the change of state produces a change in the local environment which favors the new state, the reversible sensitivity of the system to perturbing forces will be increased. This sensitivity will depend primarily upon the relaxation function of the local environment to the local change in state, and may be hundreds of times greater than Boltzmann's principle would predict for an equilibrium system. The increased sensitivity results from the fact that while the further the local environment changes in the sense favoring the existing state, the more slowly it will change in that sense, and the more rapidly it will relax in the opposing sense. If these factors closely cancel one another, high sensitivity results.

One application of this theory is to the opening and closing of ion gates in the axonal membrane, where it may explain both the high sensitivity to excitation, and Na+ inactivation. It may also provide an explanation of the extremely high electrical sensitivity of certain fish. The theory may also be involved in the maintenance of the internal chemical environment of cells, through a high sensitivity to small changes in the chemical potential gradient over a critical range.

Supported by U.S.P.H.S. grant NS 08137.

W-AM-Po70 VOLTAGE DEPENDENCE OF K-CHANNEL BLOCK BY SMALL CATIONS. Robert J. French, Jonathan J. Shoukimas and Ruthanne Mueller*, Laboratory of Biophysics, NINCDS, MBL, Woods Hole, MA 02543.

We have studied the action of six cations that cause instantaneous ($\leq$ 100 μsec) voltage-dependent block of axonal K conductance. Currents were measured immediately after a voltage step from the end of a depolarizing prepulse that turned on the conductance. To avoid variable periaxonal K accumulation during the prepulse, we performed experiments on voltage clamped, perfused squid axons with both external and internal K concentrations of 300 mM. Blocking ions (100 mM) were added to either solution. A prepulse from a holding potential of -80 mV to E = 0 mV opened the K channels without appreciable current flow. The fraction, f, of conductance remaining in the presence of a blocking ion was fitted using the equation $f = r/\{1 + \exp [s(E-E_{0.5})F/RT]\}$ with $r \simeq 1$. $E_{0.5}$ is the voltage at which 50% of the conductance is blocked. The parameter s gives a measure of the voltage dependence of the blocking reaction. For 6 ions that block when present internally, mean values of s obtained were: Li, 0.37; Na, 0.47; Cs, 0.48; glucosamine, 0.34; tetramethylammonium, 0.37; and tris(hydroxymethyl)aminomethane, 0.32. The similarity of these values suggests that these ions act at a site, or sites, in the same short region of the inner segment of the channel. For block by external Cs, s = -1.09 (negative sign indicates that block increases as voltage is made more negative inside). Thus, Cs appears to penetrate further into the membrane field from the outside than when acting from the inside. With the normal low (10 mM) K concentration externally, the effect of the internal blocking ions became more steeply voltage-dependent. Thus, the depth of penetration of these ions appears to increase as the K concentration on the opposite side of the membrane is reduced.

W-AM-Po71 THE EFFECTS OF SYMMETRICAL QUATERNARY AMMONIUM IONS OF VARYING SIZE ON K-CHANNEL CONDUCTANCE. Jonathan J. Shoukimas and Robert J. French, Laboratory of Biophysics, NINCDS, MBL, Woods Hole, MA 02543.

We have observed the effects of 5 tetraalkylammonium ions on perfused voltage-clamped squid giant axons. The ions, $N(C_nH_{2n+1})_4^+$ for n=1 to 5, have unhydrated radii, determined from Corey-Pauling-Koltun models, of 3.2, 4, 5.5, 6.7 and 7.8 Å respectively. All of these ions, when present internally, produce a voltage-dependent block of the K channel conductance. The block increases as the voltage (inside minus outside) is made more positive. At E=0, concentrations required to cause 50% steady state block for the five ions n=1 to 5 are, respectively, $\sim$ 100, 0.4, 1.2, 0.07, 0.06 mM. The action of tetramethylammonium (TMA^+, n=1) differs from that of the other ions in this series and qualitatively resembles that of the inorganic ions Li^+, Na^+ and Cs^+. With TMA^+, the block is rapid enough to cause a region of negative slope in the positive quadrant of the instantaneous current-voltage (I-E) relation. For the compounds with n=2 to 5 the block onset is slower, appearing as an inactivation-like decrease to a steady state after the rise of I_K. This has been well described for tetraethylammonium (TEA^+) and its derivatives by Armstrong (1971. J. Gen. Physiol. 58: 413). The similarities of the blocking kinetics, and of the voltage dependence of the steady state block, for the tetraalkylammonium ions, n=2 to 5, suggest that all may act at the same blocking site. The similarity in size between TEA^+ and a K^+ ion with a single hydration shell could be responsible for the blocking potency of TEA^+ being greater than that of the adjacent ions in the series - the smaller TMA^+ and the larger tetrapropylammonium. However, the results prompt one to ask what kind of channel structure could allow symmetric ions of such a wide range of radii to enter and apparently block at the same location.

W-AM-Po72 EFFECTS OF ENVIRONMENT, TEMPERATURE, AND EXCITATION WAVELENGTH ON EMISSION SPECTRA OF BACTERIORHODOPSIN. G. J. Perreault* and A. Lewis, (Intr. by B. M. Siegel) Cornell Univ., Ithaca, N. Y., 14853.

Previously, we have reported that the fluorescence spectrum of light adapted bacteriorhodopsin (bR), which has maxima at about 670, 725 and 790 nm, is dependent on excitation wavelength.[1] In this paper, we study this excitation dependence as a function of solvent environment and of temperature from 4 K to 100 K. In frozen aqueous suspensions of bR, the emission at 670 nm decreases in relative intensity with increasing excitation wavelength. In contrast, this decrease is not apparent in glycerol-water or ethylene glycol-water glasses. In all three media we observe a shift of the emission spectrum to longer wavelengths as the excitation wavelength is increased. In addition, variation in the shape of the 725 nm band suggests that it consists of more than one component.

The excitation-dependence can be most simply explained by a contribution to the emission from the K intermediate, which is the primary photochemical product. The fluorescence from this intermediate has not been identified previously. Alternately, an edge-excitation red shift (EERS) mechanism can be invoked. This would be expected in bR since EERS occurs in molecules which exhibit a large dipole-moment shift upon excitation, are embedded in a viscous polar environment, and have an excited-state lifetime which is long compared to the solvent-reorientation relaxation time.

1 G. J. Perreault and A. Lewis, Biophys. J. 21, 171a (1978).

W-AM-Po73 LIGHT INDUCED TRANSMEMBRANE PROTON FLUXES ACROSS ENVELOPE VESICLES OF HALOBACTERIUM HALOBIUM MEASURED WITH SPIN PROBES. I. Probst*, R. Mehlhorn, A. T. Quintanilha, J. Lanyi and L. Packer, Membrane Bioenergetics Group, University of California, Berkeley, California 94720 and NASA-Ames Research Center, Moffett Field, California 94035.

Two spin probes, tempamine (4-amino-2,2,6,6-tetramethyl-piperidine-N-oxyl) and carboxylate (2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl-3-carboxylic acid) that form weak base and acid anions, respectively, were used to measure light-driven transmembrane movements of protons in H. halobium cell envelope vesicles. The method requires that the outside signal of the spin probe be quenced with impermeable paramagnetic broadening agents, e.g. ferricyanide or nickel (1). The light-induced release of protons by vesicles results in an outward flow of tempamine or inward flow of the carboxylate probe. This is as expected, since only the uncharged form of the probes are permeable. In vesicles the kinetics of light-driven proton extrusion are altered when the envelopes are preloaded with Na^+. An initial rapid phase of H^+ efflux is followed by a temporary reversal of the H^+ movement; this is presumed to be a consequence of Na^+/H^+ exchange through the antiporter (2). When the vesicles are depleted of Na^+, a final pH gradient is established comparable in size to that observed with control vesicles devoid of Na^+. The spin probe method is sensitive to a Δ pH in the range of 0.01.

(1) Quintanilha, A.T. and Mehlhorn, R.J., FEBS Letters 91, 104 (1978).
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W-AM-Po74 SELF-AGGREGATION OF BACTERIORHODOPSIN IN RETINAL-RECONSTITUTED BROWN MEMBRANE. S.-B. Hwang, Y.-W. Tseng* and W. Stoeckenius (Intr. by K. A. Fisher), Dept. of Physiology and Cardiovascular Research Institute, Univ. of California, San Francisco CA 94143

Halobacterium halobium cells grown in nicotine-containing medium synthesize bacterioopsin but not bacteriorhodopsin (bR) because nicotine inhibits retinal synthesis. Bacterioopsin is found in a specific membrane fraction, brown membrane, consisting of round or oval sheets. Freeze-fracture reveals a dense particle population on one fracture face, with few particles on the other. There is no apparent order in the particle distribution and the fracture face is practically indistinguishable from the red membrane. The visible absorption spectrum of brown membrane shows two peaks at 550 nm (possibly bR) and 410 nm. CD spectra show a positive band around 540 nm and a positive and negative band around 410 nm (cross-over at 412 nm). Addition of 13-cis:all-trans retinal (50:50) to brown membrane induces a rapid increase in the 550 nm absorption band, which shifts to 560 nm after 2 min. Kinetic analysis of the absorbance increase ($t_{\frac{1}{2}}$=0.5 min, 6 min and 100 min). The typical CD bilobal pattern of purple membrane appears together with the strong negative band at 320 nm. Kinetic analysis of the ellipticity change at 590 nm shows also three kinetic constants and within 5 min 90% of the negative bond is formed. Electron microscopic observation of membrane 2 hours after reconstitution reveals structurally distinct membrane. However, X-ray diffraction studies show only weak diffused patterns similar to that of purple membrane above 80°C, where the protein crystalline lattice is dissociated. Moreover, flash-induced linear dichroism indicates bR rotates. These observations indicate that the reconstituted bR aggregates but does not form a lattice like that of purple membrane.

W-AM-Po75 THE EFFECT OF pH ON THE SURFACE CHARGES OF THE H. HALOBIUM PURPLE MEMBRANE. P. C. Mowery, S. L. Helgerson and W. Stoeckenius, Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco CA 94143

Safranine O, a cationic biological stain, was shown to bind to purple membrane sheets. The extent of binding in 10 mM tris maleate buffer varied continuously between pH 3.0 and 8.0, with a PK_a of 5.5. The titration was followed by measuring the metachromicity of the dye at 530 nm.[a] These results, when compared with complementary electron microscopic studies made by K. A. Fisher (1978, 9th International Congress on Electron Microscopy, Toronto, Vol III, pp. 521-532), are consistent with the titration of purple membrane lipids. The pH-induced aggregation of purple membrane sheets was also investigated by light-scattering at 700 nm. No changes in scattering were observed above pH 5.2 in 10 mM tris maleate or 4.7 in 10 mM phthalate buffer. The light-scattering intensity at an angle of 4^0 increased seven fold between pH 4.7 and 3.5, with a pK_a of 3.8 in phthalate buffer. This value is consistent with the titration of surface carboxyl groups at C-terminus of bacteriorhodopsin (bR). We have already reported studies on acid-induced stable species of bR (P. C. Mowery, et. al., Biophys. J., 21, 181a), and showed that a red-shifted complex bR^{acid}_{605} forms with a pK of 2.9. The data presented here show that the titration of purple membrane lipids, surface carboxyl groups on bR, and the formation of bR^{acid}_{605} are separate, distinct events. This strengthens our suggestion that bR^{acid}_{605} is formed by the titration of interior carboxyl groups.

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W-AM-Po76 THE EFFECT OF PRESSURE ON BACTERIORHODOPSIN.† H. L. Crespi and J. R. Ferraro,* Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439.

Freeze-dried samples of bacteriorhodopsin have been subjected to pressures of 3-4 kbar (3-4000 atm) in a diamond anvil cell. At this pressure, the sample changes from purple to orange (λ_{max} = 415 mm), and upon release of pressure changes to yellow (λ_{max} = 385 nm). This bleaching takes place in the dark and is at least partially reversible. Similar results have been obtained with an aqueous suspension of bacteriorhodopsin. Exchange of labile hydrogen with heavy water inhibits the observed transition and stabilizes what may be intermediate forms. These results can be interpreted in terms of a contraction of hydrophobic regions of bacteriorhodopsin followed by disruption of hydrogen bonds.

†Work was performed under the auspices of the Division of Basic Energy Sciences of the Department of Energy.

W-AM-Po77 MEASUREMENTS OF PROTON CHANGES AND BACTERIORHODOPSIN CYCLING FOLLOWING SINGLE TURNOVER LIGHT FLASHES IN PHOSPHOLIPID VESICLES. R. Govindjee*, T.G. Ebrey and A.R. Crofts*, Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

Purple membrane from Halobacterium halobium, when incorporated into egg phosphotidylcholine vesicles (pH = 6.5, 150 mM KCl) shows light-induced proton uptake. Using a pH sensitive dye p-nitrophenol and single turnover light flashes, we have measured the pH and absorption changes due to the intermediate "M" (at 412 nm). The 412 nm absorption change shows a biphasic decay. The fast phase has a half decay time of 3-5 ms, and the slow phase is ≈20 ms. The dye absorption change is monophasic with a half rise time of ≈15 ms, decays very slowly (τ >1 sec) suggesting the protons detected must cross a permeability barrier. The uptake of H^+ may be related to the slow phase of "M" decay. After a single flash, the ratio of the number of protons taken up to the number of M's formed is roughly 1. Upon addition of nigericin, all pH changes are drastically reduced. These findings suggest that the H^+ changes normally observed are due to actual translocation of H^+ across the membrane and not due to reversible binding and release on just one side of the membrane.

W-AM-Po78 CHEMICAL MODIFICATION OF BACTERIORHODOPSIN'S C-TERMINAL TAIL. Robert Renthal, Gary J. Harris*, Margaret Tse*, and Charles Bittle*. Division of Earth and Physical Sciences, University of Texas at San Antonio, San Antonio, Texas 78285

Bacteriorhodopsin (BR) spans the purple membrane (PM) with its C-terminal region extending nearly 20 amino acids out from the cytoplasmic side. We have exploited the high carboxyl group content of this C-terminal tail to cross-link BR and to attach a fluorescent probe to the cytoplasmic side of PM. Crosslinking: Reaction of PM (14 μM BR) with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.06 M) at pH 4.5 in the absence of buffer results in extensive intermolecular crosslinking of BR. Dimers, trimers, and higher polymers of BR are formed. However, pretreatment of the membrane with papain to remove the C-terminal tail almost completely suppresses the crosslinking reaction. Thus, EDC crosslinks BR molecules between the C-terminal tail and a nucleophilic site elsewhere on the membrane. At pH 5.5, the major product appears to be an intramolecular crosslink. Fluorescent labeling: PM (14 μM BR) was reacted with EDC (1 mM) at pH 5.5 in the presence of 0.6 mM dansyl hydrazine (DH). The product shows incorporation of substantial amounts of fluorescence into PM. Pretreatment with papain greatly diminishes the fluorescence incorporated. We conclude that BR is labeled by this reaction at one or more of the 5 carboxyl groups in the C-terminal tail. An additional site (or sites) is also labeled. Probably because of its proximity to retinal, its emission appears quenched by energy transfer. A recent report showed that the periodate oxidizable lipid is on the extracellular side of PM (Henderson et al. JMB 123, 259 (1978)). We have labeled this lipid by reaction with $NaIO_4$ followed by DH. These specific cytoplasmic or extracellular fluorescent labeling reactions will permit the study of surface effects on the proton pump. Supported by grants from the Robert A. Welch Foundation (AX-736) and NIH (GM 25483)

W-AM-Po79 SINGLE CHANNEL CONDUCTANCES OF BLEACHED BACTERIORHODOPSIN IN PURPLE MEMBRANE FRAGMENTS AND ITS ION SELECTIVITY IN BLACK LIPID AND LIPID IMPREGNATED FILTER MEMBRANES. Paul Shieh and Lester Packer, Membrane Bioenergetics Group, Lawrence Berkeley Laboratory, and the Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

Bacteriorhodopsin in the purple membrane of Halobacterium halobium releases protons on one side of the membrane and takes up protons from the other side when it absorbs light. We report here that bleaching of bacteriorhodopsin in the purple membrane results in the development of current fluctuations across Black Lipid and lipid impregnated filter membranes of about 10 pa/cm^2. The rise time of current fluctuation is ca. 40 msec and the decay time is 50 to 60 msec. 64% of fluctuations have a life time between 400 and 600 msec. Addition of all-trans retinal to regenerate the native pigment abolishes the current fluctuations and restores photopotentials. Current fluctuations occur with chloride salts of K^+, Rb^+, Tl^+ but not Cs^+, Li^+ and Na^+. Membrane conductance was independent of temperature between 2° to 50% in bleached preparations. These studies indicate that bacteriorhodopsin works as a proton pump in the light, and that when retinal is removed from the molecule an ionic channel forms. (Research supported by the Department of Energy.)

W-AM-Po80 QUANTUM YIELD AND ENERGETICS OF FLASH-INDUCED PROTON RELEASE BY PURPLE MEMBRANES. Donald R. Ort,* and William W. Parson (Intr. by W. Cramer), Dept. of Biochemistry, University of Washington, Seattle, Washington 98195.

Flash excitation of isolated purple membrane suspensions results in minute changes in the volume of the suspension. These occur, in part, due to the release of protons from bacteriorhodopsin to the solution. Proton movement from one buffering group to another causes a volume change if it involves a change in the number of ionic groups in the system. We studied the kinetics and quantum yield of proton movement employing a capacitor microphone to measure the volume changes. At low ionic strength (μ); one H^+ appears to be released per photochemical turnover; at high μ the ratio approaches 2. The kinetics of proton release do not parallel those of any of the transient absorption changes of the retinylidine chromophore. There is a second, slower phase of the volume change, which apparently results from the movement of a proton from one group to another within the membrane. Heating and cooling of the solution contribute to the overall volume change. Measurements of this thermal component give information of the enthalpy changes that occur during the photochemical cycle. At the point following the two steps associated with proton movements, the enthalpy of the system is about 21 kcal/mole lower than it is prior to excitation. The free energy that is stored at this point must be due to a decrease in entropy.

W-AM-Po81 CALCULATIONS OF THE VISIBLE ABSORPTION AND CIRCULAR DICHROIC SPECTRA OF THE PURPLE MEMBRANE BASED ON A SEVEN NEAREST-NEIGHBOR EXCITON MODEL. T.L. Hsiao and J.Y. Cassim, Dept. of Microbiology and Div. of Sensory Biophysics, The Ohio State University, 43210.

Previous calculations of the visible absorption and circular dichroic spectra of the purple membrane have been based on a cyclic-trimeric exciton model (1-3). Recent findings on the retinyl chromophoric positions in the membrane by neutron diffraction studies (4) and determinations of the magnitude of band splitting of the membrane visible spectra resulting from possible retinyl-retinyl interactions by linear dichroic studies (5, 6) are not in reasonable accord with predictions of this model. It will be shown that a much better agreement can be achieved with all available structural and spectral information concerning this membrane by basing calculations of the visible spectra on a seven nearest-neighbor exciton model rather than on a cyclic-trimeric one. For example, calculations based on the cyclic-trimeric model predict an approximate band splitting of 20 nm and a retinyl-retinyl distance of about 15Å. On the other hand, calculations based on the seven nearest-neighbor model predict approximately 3 to 4 nm for band splitting and 32 to 35Å for retinyl-retinyl distances which can be more readily compared to the most recent experimental findings of less than or equal to 5 nm and 22 to 58Å, respectively for these two parameters.

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W-AM-Po82 SPECTRAL ANALYSIS OF ORIENTED PURPLE MEMBRANE FILMS. D.D. Muccio and J.Y. Cassim, Department of Microbiology and Division of Sensory Biophysics, The Ohio State University, Columbus, Ohio, 43210.

The absorption and circular dichroic spectra of purple membrane films in which the plane of the membranes are oriented perpendicular to the incident beam were compared with the solution spectra in order to relate structural features of the purple membrane to a coordinate system as defined by a normal to the membrane plane and two mutually perpendicular in-plane axes. The film and solution absorption spectra were similar except for a relative depression in the 200-225 nm region of the film spectrum. The biphasic band in the solution circular dichroic spectrum was replaced by a single positive band at 555 nm in the film spectrum and the 208 nm band was completely deleted from the film spectra of the native and regenerated membranes. However, a small shoulder remained at 208 nm in the film spectrum of the bleached membrane. Although the near ultraviolet circular dichroic spectra were different, the 317 nm band remained essentially the same for both spectra. It was concluded that: (a) a relatively strong in-plane monomeric interaction occurs between the retinyl chromophore and apoprotein, (b) the helical axes of the native and regenerated membrane proteins are oriented primarily normal to the membrane plane, (c) the helical axes of the bleached membrane proteins are tilted more in-plane than the axes of the native or regenerated membrane, (d) an interaction occurs between an in-plane magnetic dipole moment of the retinyl chromophore and probably an in-plane electric dipole moment of a nearby aromatic amino acid(s), and (e) although the membrane is anisotropic with respect to coupling between electric and magnetic moments of the aromatic amino acids, the dipole moments of the aromatic amino acids are not preferentially oriented in either direction.

W-AM-Po83 STUDIES OF THE BIOSYNTHESIS, STRUCTURE AND FUNCTION OF THE BROWN MEMBRANE FROM HALOBACTERIUM HALOBIUM. G.K. Papadopoulos and J.Y. Cassim, Dept. of Microbiology and Division of Sensory Biophysics, The Ohio State University, Columbus, Ohio 43210.

The precursor of the purple membrane from Halobacterium halobium has been called the brown membrane. It is composed of two protein species, a b-type cytochrome and bacterio-opsin. We have tried to deduce the steps in the biosynthesis of the brown membrane from its components, the resulting structure and relate such features to possible function(s) of the membrane. Of the two protein components, bacterio-opsin is synthesized de novo when the oxygen tension is low. The cytochrome migrates from the red membrane into the newly assembled brown membrane. Furthermore, we find that the purple membrane is dissembled by the cells, if high oxygen tension is re-established in the growth medium. The circular dichroic (CD) spectrum of this membrane consists of two bands of opposite sign centered around 560 nm and a negative band at 317 nm similar to those observed in the spectrum of the purple membrane. However, the ellipticities of these bands, particularly the latter one, are much weaker than their counterparts in the purple membrane. Addition of retinal to the brown apomembrane results in no observable changes in the far-UV CD while in the near UV region the spectrum changes from a negligible to substantial CD which resembles that of the purple membrane. This suggests that the CD in this region may arise from a resonant interaction between the retinal and the aromatic amino acid residues. Comparison of the rate of dark adaptation at different pH values of brown and purple membranes indicates that the retinal-retinal interactions in the former are weaker than those of the latter. A model consistent with all the above observations will be proposed relating the structure and function of this membrane precursor.

W-AM-Po84 MODELS OF LIGHT DRIVEN PROTON PUMPS: COMPARISON WITH SPECTROSCOPIC STATES OF BACTERIORHODOPSIN. J.F. Nagle, and M. Mille, Departments of Physics and Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA, USA 15213.

The theory of conduction of protons along hydrogen bonded chains is used to construct models of proton pumps at a molecular level. Beginning with three different basic views of the primary event following light absorption in bacteriorhodopsin in the purple membrane of Halobacterium halobium three different models are constructed. The multiplicity of models emphasizes that there is a general family of theoretical models of proton pumps, each member of which utilizes hydrogen bonded chains. Using the theory of wavelength shifts in cromophores the intermediate states in each of the three models are compared to the spectroscopically observed intermediate states. Our model with conformational change of the retinal as the primary event is in substantial agreement with experiment.

W-AM-Po85 SPIN LABELLED, HALOGENATED, AND 5,6-DIHYDRO PIGMENT ANALOGUES OF THE PURPLE MEMBRANE. R. Crouch,* G. Renk,* R. Scott,* B. Mao,* and T.G. Ebrey (Intr. by W.C. Wise), Medical University of South Carolina, Charleston, S.C. 29403 and University of Illinois, Urbana, Ill. 61801

A spin labelled derivative of retinal [4-(2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxyl)-retinal] has been synthesized. The all-trans and 13-cis isomers combined with the apoprotein of the purple membrane of Halobacterium halobium to form pigments which are stable to hydroxylamine. The chromophore was extracted from the pigment and shown to be the intact spin labeled retinal. The electron spin resonance spectrum of the pigment showed a weakly immobilized signal as indicated by line-broadening and a slight increase in the hyperfine splitting suggesting relative freedom of the modified ring moiety in the bound state. The reduction of the nitroxide radical at low concentrations of ascorbate indicates the accessibility of the spin label to hydrophilic reducing agents. The pigment shows typical "M" formation and, when incorporated in phospholipid vesicles, has proton pumping ability. The all-trans 4-bromo- and 4-iodoretinal have been synthesized and form pigments which are blue shifted from the native bacteriorhodopsin. The results on the halogenated and spin labelled retinals, all of which contain bulky groups on the ring, indicate the binding site for the ring is somewhat nonspecific. 5,6-Dihydrodesmethylretinal also forms a pigment (λ_{max} 475 nm) which, when incorporated into phospholipid vesicles, exhibits proton pumping activity. The spectral changes of the pigment under illumination at low temperatures are comparable to those of the purple membrane.

W-AM-Po86 PHOTOSENSORY RESPONSES OF Halobacterium halobium. K.W. Foster and H.C. Berg, University of Colorado, Boulder, CO 80309.

Three dimensional tracking microscopy has been used to characterize the two independent photoresponse systems of the bipolarly flagellated rod, Halobacterium. Without stimulation, like other bacteria, they spontaneously reverse their flagella motors with constant probability. That probability is changed up or down by some chemicals and in a rate sensitive manner (adaptation) by some chemicals and both light systems. Under aerobic conditions, 10^4 cells/ml, a blue-green sensitive and non-retinal pigment keeps the cells out of the light by causing each cell to reverse more frequently to an increase of light intensity and less frequently to a decrease. The probability of reversing is proportional to the log of the step change. For step changes at physiological light intensities just below full sunlight, a step change of 3.5 results in a 5 fold increased probability of reversing. This high sensitivity to small changes and an hypothesized orientation of the chromophore in the plane of the membrane explains how the cell's long axis becomes predominately oriented parallel to the light direction. This probably gives the cell the ability to swim downward in water to avoid dangerous photooxidizing light. Under anaerobic conditions, 10^7 cells/ml or more, the cells need to use light as an energy source. They synthesize purple membrane containing bacteriorhodopsin which is able to pump protons across the membrane. This yellow-green sensitive receptor also causes more frequent reversals to a decrease in light and less frequent reversals to an increase. Consequently they aggregate in the light and orient predominately in the plane perpendicular to the light direction resulting in maximum light gathering efficiency by the in the plane of the membrane oriented retinal chromophores.

W-AM-Po87 IONIC NATURE OF PHOTOSENSORY TRANSDUCTION IN THE FLAGELLATED ALGA, EUGLENA GRACILIS. M. J. Doughty* and B. Diehn, Dept. Chemistry, Univ. Toledo, Toledo, OH 43606.

A study of the light-dependent swimming behavior of Euglena under a variety of ionic and pharmacological conditions provides a priori information on the nature of the link between blue light stimulation of this unicell and its subsequent photosensory behavior. Reduction in the light intensity incident on the cell results in a characteristic reorientation of its single locomotory flagellum with resultant tumbling behavior of the organism. The duration of this tumbling is related to the blue light intensity change. This behavioral response (a step-down photophobic response) to a standard light intensity change is unaffected by the extracellular presence of pharmacological reagents that block cation conductance across excitable membranes, but is markedly affected by monovalent cations, ouabain, anions and temperature. A23187 induces light independent tumbling behavior requiring extracellular Ca ions. Gramicidin D, under conditions where ionophore-Na membrane exchange is facilitated by a lipophilic anion, induces a similar spontaneous tumbling. The data indicate that the initial stages of photosensory transduction in Euglena involve light induced alterations in coupled monovalent cation and anion transport across the photoreceptor membrane, whilst the final stage is a Ca ion dependent alteration in the flagellar mechanochemical cycle. Such studies provide a basis for subsequent biochemical investigations of chromophore-flagellum coupling in Euglena.

W-AM-Po88 A LIGHT AMPLIFIER IN THE LENS SYSTEM OF ARTHROPOD EYES. J. J. Wolken, Carnegie-Mellon University, Pittsburgh, Pa. 15213.

Animals evolved various kinds of optical systems for imaging in the development of their eyes, from a simple pinhole to compound to refracting type optics. In most arthropod compound eyes, the optical system consists of a corneal lens (L_1) and a crystalline cone (L_2). The corneal lens is derivable from the animal's cuticle, chitin, a polysaccharide polymer. Electron microscopy of the corneal lens shows that it is structured of lamellae. In transverse section, the lamellae form a single helical spiral. In the June beetle and firefly the corneal lens (L_1) extends into the area normally occupied by the crystalline cone (L_2), described as an exocone type eye. Measurements of the corneal lens, shape, geometry, and spacings of the lamellar layets indicate that the corneal lens in these insects function to maximize their light collecting ability. Previous studies of the crystalline cone of the marine crustacean, Copilia, (Wolken, 1977) and the horseshoe crab, Limulus, (Levi-Setti et al., 1975) showed that its geometry is designed to maximize the concentration of light. Therefore the corneal lens in the June beetle and in the firefly function like that of the crystalline cone, as a light amplifier, permitting these insects to see in very dim light. To test the optical system a model was constructed of (L_1 + L_2) and photographs taken from 0 to infinity. These photographs indicated that the exposure time was reduced 8x, the image was reduced by two-thirds, but the image was greatly increased in brightness and in contrast to that of L_1 alone. (Supported in part by the Pennsylvania Lions Eye Research Foundation.)

W-AM-Po89 REGULATION OF BOVINE ROD OUTER SEGMENT PHOSPHODIESTERASE. J.B. Hurley* and T.G. Ebrey, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

Bovine ROS phosphodiesterase (PDE) can be extracted from ROS by low ionic strength buffer in the absence of Mg. In this form the specific activity of PDE decreases significantly as its concentration is raised. Protamine activates PDE at all concentrations though the specific activity still decreases at high PDE concentrations. Short trypsin treatment activates PDE over 500 fold and results in concentration independent PDE specific activity. Bleached ROS plus 0.1 mM GTP activates PDE causing the specific activity to increase dramatically at high PDE concentrations. DEAE cellulose chromatography allows separation and isolation of a PDE inhibitor from PDE. Partially purified PDE shows significantly less specific activity concentration dependence and cannot be activated by bleached ROS plus 0.1 mM GTP. A low K_m, soluble GTPase is present in bovine ROS and is activated by bleached rhodopsin. The GTPase is distinct from PDE inhibitor, and co-purifies with PDE on the DEAE cellulose column. In the presence of vesicles containing bleached rhodopsin, GTP significantly decreases the effect of PDE inhibitor on partially purified PDE. Partially purified PDE elutes from a Sephadex G-200 column with an apparent molecular weight of 340,000 and the Sephadex purified PDE shows up as a doublet at 84,000 and 88,000 on SDS polyacrylamide gel electrophoresis. A likely model in which PDE inhibitor, GTPase, GTP, and rhodopsin all play a role in PDE regulation in bovine ROS will be presented.

W-AM-Po90 THE OUTER SEGMENT PLASMA MEMBRANE REVEALS THE PIGMENT EPITHELIUM TO BE THE SOURCE OF THE 11-CIS RETINALDEHYDE IN RODS. S. Yoshikami and G. N. Nöll,* Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, Md. 20014.

The isolated and perfused rat retina, when bleached, can regenerate the rhodopsin and restore the fast photovoltage (FPV) only if 11-cis retinaldehyde is added to the Ringer. When 11-cis retinaldehyde was dispersed with the aid of liposomes into the Ringer perfusing a completely bleached rat retina, the retina took more than 2 hours ($T_{\frac{1}{2}}$=20 min) at 37 C to regenerate all of its rhodopsin, indicative mainly of regeneration in the discs, but it required only 15 min ($T_{\frac{1}{2}}$=7 min) to recover fully the amplitude of the R2 component of its FPV. 11-cis retinol and other selected retinol derivatives cannot regenerate the rhodopsin in rat or in other mammalian retinas. Such a difference in the rates of recovery of the rhodopsin and FPV occurs also in the eyes of anesthetized rats. After 95% of the rhodopsin was bleached in the live rat, its retina completely regenerated the rhodopsin in 100 min ($T_{\frac{1}{2}}$=35 min) and restored the FPV in 45 min ($T_{\frac{1}{2}}$=10 min).

The presence of rhodopsin in the plasma membrane, in addition to its role as the source of the FPV in rods, is confirmed by these experiments, when the results are considered along with the fact that the outer segment plasma membrane surrounds the disc membranes. A consequence of the anatomy of the rod outer segment membrane is, that any rhodopsin in the plasma membrane would be regenerated ahead of that in the disc, should the 11-cis retinaldehyde originate from an external source. One can thus conclude that in the mammalian eye, the all-trans retinol, a product of photolysed rhodopsin in the outer segment, leaves the retina, and returns to it from the neighboring pigment epithelium cells in the form of 11-cis retinaldehyde to regenerate the rhodopsin.

W-AM-Po91 PHOTOLYTIC SEQUENCE OF OCTOPUS RHODOPSIN AT HIGH PRESSURES STUDIED BY FLASH PHOTOMETRY. Motoyuki Tusda* (Intr. by F. Dunn) Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

The photolytic sequence of octopus (Paroctopus defleini) rhodopsin was studied by the method of rapid-scan spectrophotometry combined with flash photometry at around physiological temperature. In the sequence there may be some intermediate steps where changes in conformation of rhodopsin and solvation of rhodopsin in the membrane take place. It occurred to us that volume of activation ($\Delta V^{\neq}$) as well as heat of activation ($\Delta H^{\neq}$) and enthropy of activation ($\Delta S^{\neq}$) might be useful parameters to consider in this regard because a large $\Delta V^{\neq}$ might occur in the photolytic sequence. Thus, the effect of pressure (up to 3 kb) on the rate of conversion of several intermediate in the photolysis of octopus rhodopsin was studied. The rates of the conversion for mesorhodopsin ⟶ transient acid metarhodopsin and transient acid metarhodopsin ⟶ alkaline metarhodopsin decreased under pressure. The results show both processes had positive $\Delta V^{\neq}$, with the former transformation having the largest.

W-AM-Po92 BROAD AREA ILLUMINATION AFFECTS THE AMPLITUDE DISTRIBUTION OF DISCRETE WAVES OF LIMULUS VENTRAL PHOTORECEPTORS. Barry A. Sokol and Richard Srebro, Dept. of Ophthalmology UTHSCD, Dallas, TX 75235.

The ventral nerve of Limulus contains a variable number of photoreceptors distributed along its length. Some appear to be isolated. Others occur in clusters. When a single photoreceptor is impaled with a microelectrode and allowed to dark adapt, discrete depolarizations of variable amplitude (discrete waves, quantum bumps) are seen in the dark and their rate of occurrence increases with steady low level illuminations. Yeandle & Spiegler(J. Gen. Physiol. 61, 1973) have shown that the amplitude distribution of the discrete waves is bimodal. In their experiments the small amplitude waves were relatively insensitive to light. We consider here the possibility that small amplitude discrete waves we measure are actually large amplitude discrete waves which are being generated in other photoreceptors and these waves are measured in the impaled cell because there is some form of coupling. To test this hypothesis we recorded discrete waves under two different stimulus conditions. (1) A 40 u spot (approximately equal to one cell diameter) was centered over the impaled cell. (2) The entire nerve was uniformly illuminated. The light intensities of the two stimuli were adjusted to produce approximately equal discrete wave frequencies. The discrete wave amplitude histograms remain bimodal under both stimulus conditions. However, the amplitude of the larger discrete waves was consistently smaller under wide field illumination. In some experiments a large increase in the relative number of small discrete waves was associated with the wide field stimulus. We conclude that there is substantial coupling amongst photoreceptors along the ventral nerve which contributes to the bimodal amplitude distribution but that it is likely that both small and large discrete waves can be generated in a single photoreceptor. Supported in part by NEI grant #EY 02373.

W-AM-Po93 LOW TEMPERATURE STUDIES OF THE EARLY INTERMEDIATES OF RHODOPSIN ILLUMINATION. B. Becher. Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

The most widely accepted initial sequence of visual excitation is that rhodopsin (11-cis retinal) photoisomerizes to bathorhodopsin (all-trans) which decays to lumirhodopsin (all-trans) on warming to 170°K. However, it has been reported that at 77°K Lumi is photoconverted to Batho (Yoshizawa and Wald, Nature 197, 1279 (1963)). This result poses obvious problems to the above sequence since presumably only photoisomerization of retinal with no thermal reactions occur at 77°K. Evidence is presented that at 77°K Lumi is photoisomerized not to Batho but to a new species, L', which probably contains 11-cis retinal, and on warming is converted to Rho according to the scheme:

$$\underset{(11\text{-cis})}{\text{Rho}} \xrightleftharpoons[77°K]{h\nu} \underset{(\text{all-trans})}{\text{Batho}} \xrightarrow[170°K]{\Delta} \underset{(\text{all-trans})}{\text{Lumi}} \xrightarrow[77°K]{h\nu} \underset{(11\text{-cis})}{\text{L'}} \xrightarrow[170°K]{\Delta} \underset{(11\text{-cis})}{\text{Rho}}$$

The quantum efficiency of Lumi to L' conversion (all-trans to 11-cis retinal) is about 0.7 in contrast to 0.3 for Batho to Rho conversion. In the near-UV absorption spectrum, no significant changes are found on conversion of Rho to Batho or even to isorhodopsin (9-cis retinal) at 77°K. In contrast, photoconversion of Rho to Lumi at 170°K results in decreases in absorbance at 270 and 320 nm but not at 235 nm indicating retinal isomerization and little protein conformational change as previously reported (Ebrey and Honig, P.N.A.S. 69, 1897 (1972)). It is suggested that isomerization of retinal between Rho, Batho and Iso at 77°K is partially restricted--possibly due to the rigidity of the opsin pocket at this temperature. At 170°K, isomerization of the retinal is less constricted resulting in a measurable difference spectrum at 270 and 320 nm.

W-AM-Po94 A PICOSECOND KINETIC STUDY OF ISORHODOPSIN PHOTOCHEMISTRY. M. L. Applebury* K. S. Peters* and P. M. Rentzepis (sponsored by Robert L. Berger). Princeton University, Princeton, New Jersey 08540; Harvard University, Cambridge, Massachusetts 02138; and Bell Laboratories, Murray Hill, New Jersey 07974.

The dynamics of primary photochemical events in the photolysis of isorhodopsin have been investigated by picosecond spectroscopy techniques. Isorhodopsin, prepared from the 9-cis retinal analog and opsin, forms a red-shifted intermediate within 6 psec following photolysis at 530 nm. The kinetically generated difference spectrum of isorhodopsin and the batho intermediate has an isosbestic point near 530 nm. This suggests isorhodopsin has its own bathoisorhodopsin intermediate which differs from the batho intermediate generated upon photolysis of rhodopsin (11-cis retinal). The formation of the batho intermediate becomes thermally independent at low temperatures which is indicative of a tunnelling event. The kinetic data suggest that proton translocation is the rate determining event in the production of bathoisorhodopsin.

W-AM-Po95 PHOTOISOMERIZATION AND SALT BRIDGE CLEAVAGE: A MODEL FOR LIGHT ENERGY TRANSDUCTION IN VISUAL PIGMENTS AND BACTERIORHODOPSIN. B. Honig, Columbia Univ., N.Y., N.Y., 10025. T. G. Ebrey, Univ. of Illinois, Urbana, Illinois, 61801. U. Dinur* and M. Ottolenghi*, Hebrew Univ., Jerusalem, Israel, R. Callender, City Univ., N.Y., N.Y. 10031.

A simple model for the early events in visual pigments and bacteriorhodopsin is proposed. The model makes use of the likelihood that a negatively charged amino acid forms a salt-bridge with the positively charged nitrogen of the retinyllic chromophore. The photochemical event is a cis-trans isomerization in visual pigments and trans-cis isomerization in bacteriorhodopsin, which in each case cleaves the salt-bridge and thus separates charge in the interior of a protein. This we propose is how the energy of a photon is transduced into chemical free energy in the pigment.

The model accounts for all the characteristic properties of the primary pigments and their photoproducts. First, the extraordinary low rate of thermally populating the ground state of the primary photoproduct, as determined from psychophysical and electrophysiological measurements, is seen as resulting from the large barriers to thermal isomerization about double bonds, enhanced somewhat by electrostatic interactions in the salt-bridge. Second, the increase in enthalpy and the spectral red shift that characterize the primary photochemical events are natural consequences of the separation of charge. Proton-dependent processes detected with picosecond techniques are shown to be ground state relaxation processes following the primary photochemical event. Finally, the charged groups repositioned by photoisomerization provide a simple mechanism for vectorial proton translocation in bacteriorhodopsin.

W-AM-Po96 COMPARISON OF DISCRETE WAVES INDUCED BY LIGHT AND BY FLUORIDE IONS IN THE VENTRAL PHOTORECEPTORS OF LIMULUS.
D.W. Corson* and A. Fein, Marine Biological Laboratory, Woods Hole, MA 02543

Discrete waves can be evoked in Limulus ventral photoreceptors by both dim light and by the addition of fluoride ions to the saline bath (pH 7.0). The discrete waves evoked by the addition of 1 to 10 mM fluoride ions to the saline bath appear to be similar to the light evoked discrete waves in their time course though somewhat smaller in their average amplitude. Exposure to fluoride results in a prolongation of the response to a test flash. A bright light flash effectively adapts the fluoride-induced discrete waves. The frequency of discrete waves induced by 30 minute exposures to 1 to 10 mM fluoride increases more than linearly with concentration. Light-evoked discrete waves of similar frequency increase linearly with light intensity in this range. Fluoride-induced discrete waves appear within 15 to 20 minutes of exposure to 10 mM fluoride in saline at pH 7.0 but do not appear after an hour of exposure at pH 7.8. The fluoride effect at pH 7.0 is reversible after washing for 30 to 60 minutes in fluoride free saline. The fluoride-induced discrete waves tend to cluster together in time when the calcium concentration of the saline is reduced from 10 mM to 1 mM. While we do not know the mode of action of fluoride, it was chosen as a pharmacological agent because it is known to activate brain adenyl cyclase. We suspect that fluoride activates (or releases from inhibition) processes early in the chain of visual excitation.

W-AM-Po97 PICOSECOND KINETIC STUDIES OF AN EARLY "BLUE-SHIFTED" INTERMEDIATE IN THE PHOTOCHEMISTRY OF RHODOPSIN. M.L. Applebury*, K.S. Peters*, T. Kobayashi*, and P.M. Rentzepis, Princeton, N.J. 08540 and Murray Hill, N.J. 07974.

The kinetic appearance of primary intermediates in the photochemistry of rhodopsin have been compared for bovine and squid rhodopsin in different detergent environments. In digitonin and octylglucoside environments both bovine and squid rhodopsin show the formation of a blue-shifted intermediate with λ max near 440 nm. This blue-shifted intermediate arises with a lifetime of $\sim$ 25 psec following excitation at 530 nm and decays slowly within several hundred psec. The intermediate does not precede or correspond to formation of bathorhodopsin (prelumirhodopsin) whose risetime is < 6 psec for both bovine and squid rhodopsin in all detergent environments. Neither sonicated rod outer segments nor rhodopsin in high concentrations of ammonyx detergent give rise to this blue shifted intermediate. Kinetic data are evaluated to assess whether the species is a relevant intermediate in the direct pathway of photobleaching.

W-AM-Po98 BLUE FLASH INDUCED PROTON RELEASE IN FROG DISK MEMBRANES (FRDM) Y. Ching, Bell Laboratories, Murray Hill, New Jersey 07974 and P. A. Liebman Department of Anatomy, University of Pennsylvania+, Philadelphia, PA 19104.

Detailed kinetics studies of the blue flash induced H^+ release and accompanying rhodopsin regeneration and metarhodopsin II disappearance have been made on FRDM using absorption spectroscopy. At -2°C and pH 6.6 in isotonic solution, t1/2 = $\sim$0.6 sec. for this process. The action spectra for these spectral changes has been determined using continuous orange light for bleaching and blue flash for regeneration and confirms that the regenerated pigment is rhodopsin. The pathway of the bleaching and blue flash induced regeneration of FRDM is similar to that of bacterial rhodopsin. However, H^+ uptake and release are reversed.

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W-AM-Po99 WAVELENGTH DEPENDENT CHANGES IN SENSITIVITY RELATED TO RHODOPSIN METARHODOPSIN PHOTOINTERCONVERSION IN SCALLOP PHOTORECEPTORS. M. Carter Cornwall and A.L.F. Gorman, Dept. of Physiol., Boston Univ. Sch. of Med., Boston, Mass. 02118.

Photoreceptor cells of the distal retina of the scallop can function under high light intensity conditions while undergoing little light adaptation. The relationship between color dependent changes in photopigment states and sensitivity in these cells was studied. Pigment transitions underlying light dependent potential changes were assessed using ERP criteria. Short wavelength lights (λ<440 nM) are associated with a monophasic negative ERP whose spectral sensitivity maximum lies between 495nM and 500 nM (rhodopsin), whereas long wavelength lights (λ>660 nM) are associated with a monophasic positive ERP whose spectral sensitivity maximum lies between 575nM and 580nM (metarhodopsin). Photoisomerization of rhodopsin is associated with an increase in K^+ permeability which causes a long lasting membrane hyperpolarization. Photoregeneration of rhodopsin from metarhodopsin is associated with a decrease in light activated K^+ permeability resulting in membrane depolarization. Cells were preconditioned with deep red light so that most of the pigment was in the rhodopsin state and the percentage of the rhodopsin pigment population bleached by a step of light which produced a receptor response below saturation was determined. A 200 msec. step of light at 460nM which bleaches about 8% of rhodopsin is associated with a receptor response about 35% of maximum. At this wavelength and intensity only 6.8 seconds of illumination is required to reduce the rhodopsin population by 95%. Since we detect no rhodopsin regeneration in darkness for up to ten minutes following a bleach, these results suggest that photoregeneration of rhodopsin from metarhodopsin may be important in maintaining rhodopsin concentration and therefore sensitivity in these cells. Supported by EY01157.

W-AM-Pol00 GTP HYDROLYSIS: A POSSIBLE SOURCE OF FREE ENERGY FOR THE TRANSMITTER CYCLE IN VISUAL EXCITATION. W.E. Robinson & W.A. Hagins. Laboratory of Chemical Physics, NIAMDD, NIH, Bethesda, Md. 20014.

Signal-to-noise considerations require that many transmitter particles be released in the cytoplasm of a rod outer segment (ROS) when it responds to a single absorbed photon. Experiments with buffers for divalent metals indicate that 500-1000 Ca++ ions are released during a photon response and that these transiently reduce the Na+ permeability of the plasma membrane by reversibly binding to it. What source of free energy makes possible the release and removal of so many particles in response to a single absorbed photon? While the photon energy itself is enough to transport 1000 Ca++ ions against a small concentration gradient, we know of no system that subdivides a light quantum in this way. Could the large numerical gain required by the transmitter cycle in ROS be provided by hydrolysis of nucleotides?

The effect of light on nucleotide levels in isolated ROS has been measured. Well oxygenated dark-adapted frog retinas in 50 μM ouabain Ringers were stripped of their ROS by 10 s exposure to a hydrodynamic shear. Under infra-red illumination, the ROS suspension was filtered and aliquots were layered on silicone oil of density 1.04 in Eppendorf centrifuge tubes. The suspension in each tube was exposed to a flash that bleached 1% of its photopigment. After an incubation period, the tube was spun at 16000g. The ROS passed through the silicone oil into a drop of 0.6M perchloric acid in < 4s. The extracts were neutralized and analyzed by HPLC.

In the dark, ATP was 2-4 mM in the ROS cytoplasm and the ATP:ADP ratio was 5-8. GTP was 1.5-3 mM and the GTP:GDP ratio was ca. .80 when [Ca++] in the suspension was 1mM. Light caused disappearance of about 1 mM of extractable GDP from the ROS in < 4s. There was then a loss of 80% of the GTP within 20 s. with the appearance of an equal amount of GDP. After 60 sec there was a small increase in GMP. There was little conversion of ATP to ADP. Since the GTPase of ROS is fully activated by bleaching 0.1% of the rhodopsin (1), the observed GTP consumption in ROS is equivalent to more than 500 molecules per absorbed photon in 20 s. Thus the GTPase is active enough in isolated ROS to power the transmitter cycle in visual excitation.

(1) W.E. Robinson & W.A. Hagins, Biophys. J. 17, 196a (1977)

W-AM-Pol01 IS MORE THAN ONE PROTON PUMPED IN THE BACTERIORHODOPSIN PHOTOCYCLE? R.A. Bogomolni R.H. Lozier, G. Sivorinovsky* and W. Stoeckenius. (Intr. by S.B. Hwang) C.V.R.I. and Depart. of Biochemistry and Biophysics, University of California, San Francisco, California 94143

We measured the quantum yield for proton pumping (QY_H+) in H. halobium cells. Coupled proton influxes, presumably associated with preexisting gradients, were minimized by low pH(5.0) or by treatment with uncouplers, nigericin, or the ATPase inhibitor DCCD. Incident light flux was measured by chemical actinometry. The fraction of light absorbed by bacteriorhodopsin(bR) was calculated from the total absorption(measured in an integrating sphere) using bR concentrations determined by established methods. Values of QY_H+ calculated from initial rates of light induced acidification range from 0.45 to 0.63 proton per photon. The ratio between QY_H+ and the quantum yield for cycling(QYcy) is the number of protons pumped per cycle. Values near 0.3 and 0.79 for QYcy have been reported for native(at low or high ionic strength) and for ether-treated purple membrane in high salt respectively. We found, however, that the relative photocycling yield is similar under all those conditions(see Lozier et al. abstract)although its absolute value has not been determined yet. If QYcy is also the same under physiological conditions, a QYcy=0.3 combined with $QY_H+ \cong 0.5$ implies that near two protons are pumped by bR per photocycle. We found that net proton release in aqueous purple membrane suspensions, induced either by constant light or by light flashes gives the identical result $H^+/bR \leq 1$. However, at high ionic strength the net proton release doubles indicating that more than one proton may be released per cycle, although it cannot be shown in this system that every proton released is pumped. The implication that more than one proton is pumped per cycle, if true, will significantly affect our view of the pumping mechanism

W-AM-Pol02 BACTERIORHODOPSIN MONOMERS PUMP PROTONS. Norbert A. Dencher and Maarten P. Heyn, Biozentrum, University of Basel, CH-4056 Basel, Switzerland.

Bacteriorhodopsin can be incorporated into large unilamellar phosphatidylcholine vesicles by a dialysis method starting with monomerically solubilized bacteriorhodopsin. In previous work it was shown[1] that at temperatures below the lipid phase transition, bacteriorhodopsin crystallizes into patches with the same hexagonal lattice as is observed in the purple membrane. Above the phase transition, the lattice disaggregates and the protein molecules are monomeric. This reconstituted system thus allows us to study the function and properties of bacteriorhodopsin as a function of its state of aggregation by simply changing the temperature. One major question is whether the hexagonal state of aggregation is required for proper functioning of the light-driven proton pump. Our experiments show that proton translocation occurs not only in the crystalline state but in the monomeric state as well. Data will be presented on the photocycle and on the kinetics of the light-induced pH response in the two states of aggregation. (Supported by the Swiss National Science Foundation grant 3.333-0.78 (M. P. H.) and by an EMBO Fellowship (N. A. D.)).

1. R. J. Cherry, U. Müller, R. Henderson and M. P. Heyn, J. Mol. Biol. (1978) 121, 283.