W-AM-1 BIOLUMINESCENCE. J.W. Hastings, The Biological Laboratories, Harvard University, Cambridge, Mass. 02138.

Bioluminescence is sometimes displayed in spectacular ways and exhibits diversity in mode of display, molecular mechanisms and cellular control. It involves an enzyme-mediated chemiluminescence, an exergonic (usually oxidative) chemical reaction in which part of the energy is used to populate an excited state of an intermediate or product molecule thereby converting chemical to radiant energy. The color of bioluminescence differs in different species, a fact presumably related to function, but also signaling underlying biochemical differences. Recent work on chemiluminescence has provided new insights concerning reactions in bioluminescence. Already, it is evident that not only are different specific molecules involved, it is likely that there is more than one prototype of chemical mechanisms whereby the excited state is populated. Thus one must emphasize that luciferin (substrate) and luciferase (enzyme) are chemically different in different groups, that the terms are generic for bioluminescence, irrespective of what the chemical structures or mechanisms may be. In this connection it is worth pointing out that ATP is involved in only one system. The control of bioluminescence occurs at different levels. At the cellular level flashing is linked to action potentials, but the biochemical coupling differs. In coelenterates, reaction of calcium with a precharged photoprotein is postulated; in dinoflagellates there is evidence that a pH jump triggers the flash. The many different uses of light emission (in different organisms) fall under one or another of three major headings (1) to aid in predation (2) to deter or escape from predators (3) communication. In organisms such as fungi where no biological use of the light is evident, the system may function at the biochemical level by providing chemically generated excited states.

W-AM-2 MODELS FOR LIQUID PHASE CHEMILUMINESCENCE. Thérèse Wilson*, The Biological Laboratories, Harvard University, Cambridge, Mass. 02138.

Two models have received particular interest recently: the transfer of one electron from a powerful oxidant to a reductant and the unimolecular cleavage of four-membered ring peroxides (dioxetanes). The emphasis will be on the results obtained with simple synthetic dioxetanes because of the involvement of the oxygen molecule as in bioluminescence. Among the topics discussed will be: a) requirements for efficient chemical generation of excited states (exoergicity, availability of excited state in product, little structural change during reaction); b) influence of chemical structure on yields and multiplicity of excited products (all simple dioxetanes generate excited products in yields up to 50%, but triplets states are generally favored over singlets); c) role and mechanisms of energy transfer (for example, for detecting and monitoring triplet products); d) relation with bioluminescence.

W-AM-3 CONTROL OF LIGHT EMISSION IN THE SYMBIOTIC LUMINOUS BACTERIA.
K.H. Nealson. Scripps Institution of Oceanography, La Jolla, Cal. 92037

Luminous bacteria can be found free floating in the ocean or as associated forms; either as saprophytes or as true symbionts. In the latter case they are found in highly specialized luminous organs where they act as a light source for the co-symbiont fish or squid. In contrast to the more familiar bursts or flashes of light typically associated with higher organisms, the bacterial emission is a continuous one, remaining bright for the life of the co-symbiont fish. Despite the fact that a continuous emission occurs, a complex control system has been shown to occur in the luminous bacteria. Firstly, the synthesis of bacterial luciferase is under a control called autoinduction (Nealson,Platt & Hastings,J.Bact. 1970). This autoinduction is mediated through a small diffusable activator molecule synthesized by the luminous bacteria themselves. When this activator, which is continuously produced by the cells, accumulates in the medium to a critical concentration, the synthesis of the components involved in bioluminescence is induced. Secondly, the level of luciferase per cell is very sensitive to the level of oxygenation of the medium. Those bacteria isolated as symbionts exhibit a great increase in luciferase activity per cell at low oxygen levels. Finally, the production of extracellular metabolites can greatly effect the *in vivo* activity of the bacteria. The accumulation of large amounts of organic acids effects both the growth of the bacteria and their ability to emit light. Symbionts typically excrete copious quantities of oxalacetate and pyruvate which must be buffered or removed is light emission is to continue. The features of the controls discussed above are consistent with the hypothesis that the symbiotic luminous bacteria are very highly adapted for the niche they fill and the function they perform.

W-AM-4 COELENTERATE BIOLUMINESCENCE: CALCIUM ACTIVATED PHOTOPROTEINS AND ENERGY TRANSFER IN BIOLUMINESCENCE.
J. G. Morin*, Department of Biology, University of California at Los Angeles, Los Angeles, California 90024 (Intr. by J. W. Hastings).

Bioluminescence is known from all of the classes and subclasses of the Cnidaria and Ctenophora. Biochemically the luminescence in each of these groups is similar. From these forms a single light emitting molecule with a molecular weight of about 30,000 can be extracted which is specifically sensitive to Ca^{++}, even in the absence of O_2. These "calcium activated photoproteins" (CAP's) are the chemical intermediate (a complex of O_2, luciferin, and luciferase) in an oxidative reaction which will proceed to completion only in the presence of Ca^{++}. The emission is spectrally broad (λ_{max}=460 to 490nm depending on the species). The luciferin has been partially synthesized (Cormier's laboratory) and contains a pyrazine ring and an imidazole ring. In many luminescent Hydrozoa and Alcyonaria, but not the Zoantharia, Scyphozoa or Ctenophora, there is an accessory molecule, the green fluorescent protein (GFP), which functions as an energy transfer system in the luminescent reaction. The energy transfered from the CAP to the GFP is emitted as a fluorescence with a narrow band width (λ_{max}=508 to 518nm). The presence of these GFP's allows the luminescent cells (photocytes) to be visualized by using fluorescence microscopic methods. The photocytes are homogenously fluorescent, are endodermal, usually have long dendritic-like processes, and the distribution is species specific. The photocytes are regulated by systems which may be 1) through-conducting and give a unitary response to each stimulus, 2) incrementally conducting and respond repetitively to each stimulus, 3) non-conducted and local, or 4) extracellular. Depending on the species the duration of the flash varies from about 50 msec to over 1 sec.

W-AM-5 NEURAL REGULATION OF BIOLUMINESCENCE. J.F. Case, D. Oertel, M. Anctil and A.T. Barnes, Department of Biological Sciences, University of California, Santa Barbara, California 93106.

Light organs of firefly larvae and adults and of two types of fish, midshipmen and lanternfish, will be discussed as examples of neurally controlled luminescent tissues. Firefly light organs and fish photophores, but not the caudal organs of lanternfish, have alpha adrenergic innervation expressed in a wide variety of synaptic ultrastructure and light emitting characteristics. "Slow" light organs, firefly larval and fish photophores, are characterized by either uncomplex terminals directly innervating photocytes or by neurosecretory terminals on an immediately adjacent sinus. "Fast" light organs, firefly adult and lanternfish caudal organ, have either an intermediate cell between an uncomplex terminal and the photocyte or an elaborate terminal with a large surface of contact with the photocyte. Possible excitation mechanisms will be considered in the context of these structural limitations and the biochemistry and physiology of luminescence in these organisms.

W-POS-A1 OBSERVATIONS ON A STOICHIOMETRIC INTERACTION OF LYSOLECITHIN AND CHOLESTEROL AND ON THE NATURE OF THE PREMELT TRANSITION OF DIPALMITOYL-LECITHIN (DPL). R.P. Rand, D. Randlett*, Brock University, Canada; D. Purdon* and D.O. Tinker*, Toronto University, Canada; and K. Larsson*, Goteborg University, Sweden.

A premelt transition at 35°C is observed calorimetrically before the chains of DPL melt at 44°C to give the Lα lamellar phase in water. This premelt transition has been shown to be a change in orientation of the crystalline chains from tilted (Lβ' phase) to perpendicular (Lβ phase) to the bilayer plane. Three distinct activities of phospholipase A_2 have been correlated with these three phases.

Lysolecithin and cholesterol have been shown to combine stoichiometrically in water in the ratio 1/1 forming a lamellar phase separate from excess lysolecithin or cholesterol or water, but not from egg lecithin. The lowering of the electrical resistance of lecithin thin films by lysolecithin is reversed by cholesterol. These results indicate that a stable complex is formed between cholesterol and lysolecithin suggesting a stabilizing role for cholesterol in membranes. Also, such interactions may be important in the early events of atherosclerosis where lysolecithin levels increase five-fold.

W-POS-A2 DIFFUSION IN MEMBRANES: FLUORESCENCE QUENCHING MEASUREMENTS. M. Andrich* and C.S. Owen*, Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174. (Intr. by W.D. Bonner)

Diffusion within lipid bilayer membranes occurs on a time scale of nanoseconds which is within the resolution of existing fluorescence lifetime measurement technology. Theoretical equations have been derived which describe the decay of fluorescence due to diffusion-dependent quenching in the "two-dimensional" case where particle motion is confined to a thin lipid bilayer. The decay of emission following a short exciting pulse deviates from strict exponential decay if time dependent collisional quenching of the excited fluorophores occurs. The curvature of the trace of log I intensity versus time then depends upon the diffusion constant for motion in the membrane and the molecular size. The curves resulting from the two dimensional theory are compared with the usual theory which is based on the Smoluchowski spherically symmetric boundary conditions. Experiments have been carried out using the long lived fluorophore pyrene in sonicated lipid vesicles. Measurements have been made in which pyrene is quenched by quinone (Q4) and by pyrene self-quenching (through excimer formation). A variety of temperatures and reactant concentrations were employed and then analyzed in terms of the theory. From this analysis one can infer a value for the diffusion constant D which characterizes lateral mobility in the plane of the membrane. (Supported by NIH Fellowship GM55064 and by Prof. Britton Chance under NIH grant GM12202.)

W-POS-A3 PROPERTIES OF YEAST CELL OSCILLATIONS. J. Aldridge*, J. Higgins, and E.K. Pye. Department of Biochemistry, Medical School, University of Pennsylvania, Philadelphia, Pennsylvania 19174.

Oscillatory metabolism has been well described in yeast. Recent data will be presented showing that cell populations of the yeast S. carlsbergensis exhibit more complex behavior. By monitoring intracellular NADH during oscillatory glycolysis, one observes that: 1- oscillatory behavior only occurs beyond a critical cell density; 2- a freely diffusible compound affects mutual synchronization; and 3- small changes in external ion concentrations can, as a function of phase, reversibly shift the system from an oscillatory state to a non-oscillatory one. In addition, there is evidence for more than one metabolic state in the absence of any ion. It is well known that coupled non-linear systems have the potential to express these phenomena. In the past, glycolytic oscillations have been explained in terms of non-linear metabolic feedback. By coupling models of glycolytic metabolism, reasonable success has been achieved in explaining the experimental behavior. The merits of these models will be discussed.

W-POS-A4 COMPLEMENT INDUCED STEP CONDUCTANCES IN BILAYER MEMBRANES. Darold Wobschall and Catherine McKeon*, State University of New York at Buffalo, Buffalo, N.Y. 14226.

Increases in the electrical conductance of lipid bilayer membranes was observed upon the addition of antibody (Ab), antigen (Ag), and complement to the adjacent aqueous solution, confirming the reports of del Castillo et al. and Barfort et al.[†] At low concentrations nonuniform step changes (initially increases) in the conductance are observed. Following the onset of these steps a voltage-dependent conductance noise is observed. Membrane rupture is frequent especially at higher concentrations. It is postulated that the conductance increase is a result of a hole in the membrane produced by the Ab-Ag activated complement attack, somewhat similar to cell membrane lysis. Measurements of the step size as a function of salt concentration are consistent with an average pore diameter of 12 to 22 Å.
(This research was supported by an NIH grant).

[†]P. Barfort, E. Arquilla, P. Vogelhut, Science, 160, 1119 (1968).

W-POS-A5 REGULATION OF CALCIUM TRANSPORT IN B. MEGATERIUM. E. E. Golub,* William C. Nash,* and Felix Bronner, Department of Oral Biology, The University of Connecticut Health Center, Farmington, Connecticut 06032.

Whole cells of B. megaterium in the vegetative phase, while exhibiting ^{14}C-glutamate uptake in the presence of K ascorbate and phenazine methosulfate when assayed in imidazole buffer (pH 6.6), exhibit Ca extrusion. Vesicles prepared from these cells show energy-dependent uptake of both glutamate and calcium. However, studies with surface marker enzymes, detailed comparison of calcium and glutamate uptake by vesicles in various buffers and under different lysis conditions, as well as comparative experiments with lysozyme and French press-derived vesicles, indicate that the vesicle population is heterogeneous and that calcium transporting vesicles are inside out, while glutamate transporting ones are right side out. The calcium transporting vesicles may therefore be part of a cellular calcium extrusion mechanism. Since sporulating cells accumulate calcium, we imagined the calcium extrusion system to be repressed during sporulation. However, it proved possible to prepare calcium transporting vesicles from sporulating cells and to demonstrate energy-dependent extrusion in whole cells under conditions of net calcium accumulation. Hence calcium uptake during sporulation must involve a system separate from the efflux mechanism. Moreover, the expression of calcium uptake and the repression of calcium extrusion may be linked to the changes in energy economy associated with sporulation. (Support by NIH, NSF and U. Conn. Research Foundation).

W-POS-A6 THE NATURE OF THE CHOLINERGIC RESPONSE IN ARTIFICIAL MEMBRANES CONTAINING A PROTEOLIPID FROM ELECTROPHORUS. M. Parisi, N.Adragna,* P.Salas* and R.Spiguel.* Cátedra de Física Biomédica. Facultad de Medicina, Univ. de Buenos Aires, Argentina.

Previous work has demonstrated that artificial lipidic membranes containing proteolipids from cholinergic tissues develop special properties, including a "cholinergic conductance" that can be blocked by curare. New experiments were now performed to clarify the mechanism underlying this phenomenon. Artificial membranes separating two aqueous phases were made as previously described. The permeability properties of membranes containing natural lipids and proteolipids from cholinergic tissues were compared with membranes containing synthetic lipids alone. Different salt gradients were established increasing salt concentration on one side. In the proteolipid-containing membrane diffusion potentials indicating cationic selectivity were observed. For a 10 fold salt gradient the observed diffusion potentials were in each case: KCl, 12,6$\pm$2,0 mV; NaCl 16,2 mV $\pm$3,2; acetylcholine-Cl 60,1$\pm$5,0 mV. These properties were not observed in pure lipid membranes with similar lipid composition indicating that proteolipids are responsible for the reported result. This cationic selectivy shown by the proteolipid containing membranes was abolished by d-tubo-curare 10^{-4} M in the bath. It can be concluded that membranes containing hydrophobic proteins from cholinergic tissues develop cationic selectivity. Apparently, diffusion potentials indicates a greater permeability for acetylcholine, as compared with Na and K.

W-POS-A7 A SIMPLE NETWORK MODEL FOR ISOTONIC TRANSPORT IN EPITHELIA.
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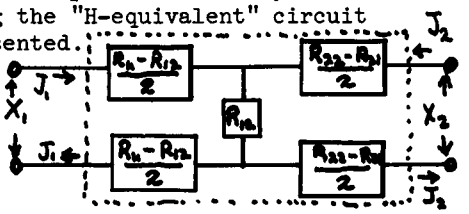
Two versions of network thermodynamics are available for modeling complicated biological systems. (Peusner, Ph.D. Thesis, Harvard Biophysical Lab. 1970. Oster, Perelson and Katchalsky, Nature 234:393 (1971)), the first uses ordinary electrical circuit notation while the second introduces bond graph notation. Both have distinct advantages over models using equations alone. The model for isotonic transport introduced by Curran and MacIntosh (Nature 193:347 (1962)) and applied to biological epithelia by Diamond (J. Gen. Physiol. 48:15 (1964)), Curran and others, is modeled by Peusner's method. In Peusner's thesis, a "T-equivalent" circuit was developed to represent two-flow, coupled energy conversion processes after the equations of nonequilibrium thermodynamics. This T-equivalent circuit is not an adequate representation of these processes as it does not preserve the identity of the individual flows on the output side. In this model an "H-equivalent" circuit is introduced to represent coupled salt and water flow and active salt transport is modeled as an ideal current source. Means of making the model more detailed to incorporate additional information and comparison with compartmental analysis will be discussed. The rational for substituting the "H-equivalent" circuit for Peusner's "T-equivalent" will be presented.

$$X_1 = R_{11} J_1 + R_{12} J_2$$

$$X_2 = R_{21} J_1 + R_{22} J_2$$



W-POS-A8 ELECTRICAL BREAKDOWN OF LIPID BILAYERS AS AN ELECTROMECHANICAL INSTABILITY. Joseph M. Crowley, Department of Electrical Engineering, University of Illinois, Urbana, Illinois 61801.

The lipid bilayer is modeled as a bulk elastic layer subject to compressive electric forces caused by applied voltages. Analysis of this model shows that a compressive instability develops when the electric stress exceeds a critical value. This instability tends to crush the film and thus rupture it. The predicted breakdown voltage, when compared with measured values for phosphatidylcholine and cholesterol, shows fair agreement, considering the uncertainty in the estimate of elastic parameters

W-POS-A9 INTERACTION OF AMPHOTERICIN B WITH LIPOSOMES: EFFECT OF CHOLESTEROL.

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Sodium 22 efflux was measured in unsonicated liposomes, composed of egg lecithin, dicetylphosphate, and cholesterol (chol), both in the presence and absence of amphotericin B (amph). The concentration of amph used was 2.5 μg/ml. With bilayer sterol concentrations up to 20 moles%, amph-mediated Na 22 efflux progressively increased. Chol concentrations in excess of this decreased Na 22 transport. This relationship between sterol content and amph-mediated Na 22 efflux was independent of surface charge since the same phenomenon was observed in liposomes made positively charged with stearylamine. Increasing the concentration of amph in the aqueous phase (from 2.5 to 12.5 μg/ml) further enhanced Na 22 permeability in low (5 moles%) and high (45 moles%) chol containing liposomes, but not in vesicles with a sterol content of 20 moles%. Dibucaine and propranolol, two local anesthetics which disrupt phospholipid-sterol interactions, increased amph-mediated Na 22 transport only in the high chol liposomes. In the absence of amph, chol containing vesicles become more permeable as the temperature is raised. In the presence of amph, these same liposomes display either no change or a slight decrease in Na 22 efflux over the same temperature range, indicating a different transport mechanism. These observations indicate that amph channels form with difficulty in liposomes containing approximately equimolar amounts of chol and lecithin. Optimum conditions for amph-mediated Na 22 efflux occur at about 20 moles% sterol. A high chol content might impede channel formation either by reducing amph-sterol interactions (chol-chol contacts would occur in these vesicles) or by reorganizing the bilayer such that amph cannot assume the proper orientation.

W-POS-A10 OXYGEN DIFFUSION IN BIOLOGICAL MEMBRANES AND PHOSPHOLIPID DISPERSIONS. J.M. Vanderkooi and S. Fischkoff*, Johnson Research Foundation, Department of Biophysics and Physical Biochemistry, University of Pennsylvania, Philadelphia, Pa. 19174

Determination of the oxygen diffusion constant in membranes is afforded by use of a diffusion-limited reaction between the singlet excited state of the fluorescent probe pyrene located in the membrane and paramagnetic molecular oxygen. The fluorescence intensity and lifetime of pyrene in both natural and artificial membranes decreases about 80% in the presence of 1 atm O_2, while the fluorescence excitation and emission spectra are unaltered. Assuming the oxygen partition coefficient between the membrane and aqueous phases to be 4.4, the diffusion coefficients for oxygen at 37°C are 1.5×10^{-5} cm^2/sec in dimyristoyl lecithin vesicles, 9.32×10^{-6} cm^2/sec in dipalmitoyl lecithin and 7.27×10^{-6} cm^2/sec in erythrocyte plasma membranes. The heats of activation for oxygen diffusion are under 3 kcal per mole ; however, a dramatic increase in the diffusion coefficient occurs at the phase transition of dimyristoyl lecithin and dipalmitoyl lecithin. The oxygen diffusion coefficients are 2-3 orders of magnitude larger than diffusion coefficients of aromatic hydrocarbons in membranes; thus, oxygen diffusion shows marked deviation from that predicted by the Stokes-Einstein relationship. Supported by USPHS GM 12202, NINDS 10939-01 and SCOR Brant HL 15061-03.

W-POS-A11 THE DEPENDENCE OF Ca EFFLUX FROM SQUID AXONS ON $[Na]_i$ AND $[Ca]_i$. L. J. Mullins, S. Spangler*, and F. J. Brinley, Jr. Dept. of Biophysics, University of Maryland School of Medicine, Baltimore, Md. 21201 and Dept. of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md. 21205

Squid axons were pretreated with CN, and dialyzed with an internal solution containing the K salts of isethionic and D-aspartic acids, 190 mM each, glycine 275 mM and TES 10 mM to buffer the axoplasm to pH 7. EGTA/CaEGTA mixtures containing ^{45}Ca were then added to the dialysis fluid at a total concentration (EGTA + CaEGTA) of 1 mM. The mixtures were prepared such that ionized [Ca] varied from 0.004-11 μM. Measurements were made at 12 different values of $[Ca]_i$ within the range cited above; the results were that Ca efflux saturates at a $[Ca]_i$ of about 0.5 μM where Ca efflux into Na seawater is ~0.5 pmole/cm^{2}sec and is about half maximal at a $[Ca]_i$ of 0.3 μM. Ca efflux is a linear function of $[Ca]_i$ from 0.004-0.05 μM. The above measurements were made with $[Na]_i$ = 2 mM. When $[Na]_i$ was increased to 40 or 80 mM, Ca efflux declined. This change was found for all $[Ca]_i$ studied (0.03-0.62 μM). About 40 mM $[Na]_i$ decreased Ca efflux to 1/2 of control values and the decrease appeared to be directly related to the decrease in the Na gradient (E_{Na} - E_m) produced by increases in $[Na]_i$. [Aided by grants from NINDS (5846 & 8336) and NSF (GB41593).

W-POS-A12 METABOLIC AND GENETIC CONTROL VIA UNIDIRECTIONAL *IN VIVO* MOTIONS. Sydney J. Webb, Department of Physics, University of South Florida, Tampa, Florida 33620

Three separate but integrated lines of research have suggested that in any given cell type a specific overall unidirectional motion of its component parts occurs. First, when microbial cells adjust to a new nutritional environment one daughter cell only, after their first division, possesses the molecular anatomy conducive to the new nutrition. Second, as cells progress through their life-span, light between 320-400 nm, absorbed by a component of the membrane respiratory chain, sequentially mutates each of the genes in the same sequence as the genes are present in DNA. The direction in which the sequence of mutations occurs and the starting point both are controlled by the carbon source supplied. Third, specific frequencies of microwaves, between 2 and 200 GH_z, are able to affect cell proliferation and a variety of metabolic events. The effective frequencies form distinct series in which each effective frequency is separated from the next by a constant frequency; the effective frequencies and the value of their constant separation both are controlled by nutritional factors. These findings suggest that a oneway motion of old and new cell components occurs *in vivo* which is energized by a rotational motion of metabolites as they are used or produced. Thus the overall motion seems to be a spiral one with its pitch controlled by the angle and size of a rolling or flip-flop motion executed by metabolites. This motion is considered to control what events may occur and the time and place of their occurrance.

W-POS-A13 EFFECTS OF PARAMAGNETIC SHIFT REAGENTS ON THE NMR SPECTRA OF EGG PHOSPHATIDYLCHOLINE ENRICHED WITH ^{13}C IN THE N-METHYL CARBONS. Barry Sears, William C. Hutton*, and T.E. Thompson, Department of Medicine, Boston University School of Medicine, Boston, Mass. 02118, and the Departments of Chemistry and Biochemistry, University of Virginia, Charlottesville, Virginia, 22901.

Effects of paramagnetic shift reagents on the ^{13}C NMR spectra obtained from single-walled vesicle dispersions of egg phosphatidylcholine enriched with ^{13}C in the N-methyl carbons have been investigated. Spectra obtained at 25 MHz show that Yb^{+3} at Yb to lipid phosphorus ratios as low as 1/12 causes complete resolution of the N-methyl carbon resonances from molecules on the inner and outer faces of the vesicle bilayer. No precipitation of the vesicles is caused by Yb^{+3} at these concentrations nor is appreciable line broadening observed. T_1 measurements of the resonances separated by Yb^{+3} indicate that the choline groups on the inner bilayer surface are less mobile than are the same groups in the outer surface. Gated proton decoupling measurements, which show that the NOE is 2.9 ± 0.1, indicate that the dominant mode of relaxation is dipolar interaction. Other paramagnetic shift reagents frequently used in proton NMR investigations of phosphatidylcholine vesicles do not give complete separation of the N-methyl ^{13}C signals from the two bilayer surfaces. $K_3Fe(CN)_6$, Eu^{+3} and Pr^{+3} cause precipitation of the phosphatidylcholine vesicles at concentrations which give only incomplete resolution of these signals. This work was supported by NIH Grant GM-14628.

W-POS-A14 PROTON NMR STUDY OF SPHINGOMYELIN IN BILAYER VESICLES. Y. Barenholz* and T.E. Thompson, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Va. 22901.

PMR studies of single-wall vesicles, homogeneous in size, comprised of sphingomyelin or mixtures of sphingomyelin and phosphatidylcholine have been carried out with an FT 100 MHz spectrometer. T_1 values obtained at 37° for beef sphingomyelin N-methyl, methylene and terminal methyl protons are much smaller than the corresponding values reported for phosphatidylcholines. Although both beef sphingomyelin and dipalmitoyl phosphatidylcholine undergo phase transitions within the temperature interval studied, the dependence of T_1 values on temperature is smaller for sphingomyelin. This temperature dependence is also smaller for sphingomyelin than for egg phosphatidylcholine. T_1 values for the N-methyl proton resonances of sphingomyelin show the least temperature dependence. In the absence of paramagnetic shift reagents, the N-methyl proton signal originating from the outer surface of sphingomyelin vesicle bilayers is separated by about 5 Hz from that originating from the inner surface. If the shift is increased by Pr^{+3}, it is apparent that T_1 values for the inner surface N-methyl protons are smaller than the corresponding values for the outer surface groups. N-methyl deutero-egg phosphatidylcholine in vesicles of mixed composition reduces the splitting of the sphingomyelin N-methyl proton signal. Using Pr^{+3}, the ratio of the number of molecules of sphingomyelin in the inner bilayer surface to that in the outer surface is found to be independent of the mole fraction of phosphatidylcholine in vesicles of mixed composition. These studies were undertaken to seek a physical basis for the apparent interdependence of phosphatidylcholine and sphingomyelin compositions seen in mammalian membranes. Supported by NIH Grant GM 14628.

W-POS-A15 IN VITRO INHIBITION OF E. COLI GTP CYCLOHYDROLASE BY SEVERAL REDUCED FOLATES. J.C. Nixon, R.F. Butz*, C.A. Nichol*, I.K. Dev* and R.J. Harvey,*Departments of Medicinal Biochemistry and Microbiology, Wellcome Research Laboratories, Research Triangle Park,N.C. 27709.

GTP cyclohydrolase has been isolated from E. coli B cells by ammonium sulfate fractionation, Sephadex G 200 chromatography and affinity chromatography. When preincubated separately with GTP cyclohydrolase, N^5-methyltetrahydrofolate, N^{10}-formyl tetrahydrofolate and dihydrofolate markedly inhibit the capability of the enzyme to convert 8-^{14}C-GTP to neopterin triphosphate as monitored by ^{14}C-formate released. When incubated together, N^{10}-formyl tetrahydrofolate and dihydrofolate appear to bind to separate sites on GTP cyclohydrolase and exhibit an independent inhibitory affect. We observed that GTP cyclohydrolase activity was inhibited by 50% at a tetrahydrofolate concentration about 8×10^{-5}M. Likewise, N^5-methyltetrahydrofolate, N^{10}-formyl tetrahydrofolate and dihydrofolate inhibit the activity of GTP cyclohydrolase by 50% between 2×10^{-5} and 2×10^{-4}M. Since this concentration is within the range reported for intracellular levels of folate cofactors, it would indicate that the inhibition observed in vitro may reflect feedback control by folates of GTP cyclohydrolase deserving study in vivo.

W-POS-A16 ELECTRICAL CHARACTERISTICS OF EPITHELIAL CELL MEMBRANES IN NECTURUS GALL-BLADDER (GB). L. Reuss and A.L. Finn, Department of Medicine, Univ. of North Carolina, Chapel Hill, N.C. 27514.

Microelectrode techniques were employed to determine the effects of ionic substitutions in the bathing solutions on membrane potentials and resistances in Necturus GB epithelium. With Ringer solution on both sides, the mucosal membrane potential is 56 mV, the serosal membrane potential 58 mV (cell negative to both solutions), and the transepithelial potential -2 mV (referred to serosa). The shunt resistance is 5% of the transcellular resistance. From these values, the equivalent electromotive forces at the mucosal and serosal cell borders were calculated to be 41 and 69 mV respectively, both oriented with the negative pole toward the cell interior. Ionic substitutions on the serosal side show that the basal-lateral membrane is K permselective ($P_{Na}:P_{Cl}:P_K$ = .07:.15:1.0, where $\Delta PD/\Delta K$ was 54 mV/10-fold change). The mucosal cell border also exhibits high K and low Na permeability, as evidenced by: hyperpolarization with external K reduction, depolarization with high K (slope, 42 mV/10-fold change), moderate hyperpolarization when Na is replaced by choline. The relative permeabilities are $P_{Na}:P_{Cl}:P_K$ = .15:.40:1.0. High K in the mucosal solution reversibly decreases the apical membrane resistance, while substitution of Na and K with choline produces the opposite effect. As the GB mainly transports an isotonic NaCl solution in the mucosa-to-serosa direction, these results are compatible with the existance of two parallel pumps: first, a neutral NaCl mucosal entry step in series with a similar serosal exit step (both being independent of the electrical potential), and second, Na-K exchange pumps at each border which, as in non-polar cells, maintain cation content and hence membrane potentials.

W-POS-A17 THE EFFECTS OF SURFACE CHARGE AND THE DOUBLE LAYER ON THE RATE OF SURFACE-CATALYZED ENZYMATIC REACTIONS. John A. DeSimone, Department of Physiology, Medical College of Virginia, Richmond, Virginia 23229.

When an enzymatic reaction occuring at a charged surface involves charged substrates and products, the rate of transport to the surface and hence, the rate of reaction, will be greatly influenced by the electric field near the surface. The field may either augment or retard the reaction rate relative to a hypothetical uncharged state. A calculation is made based upon the Nernst-Planck equations for substrate, product and one inert ionic species. In the first case the effects of the double layer are ignored. In the second case the effects of the double layer are included and the contribution of the reacting species is explicitly considered in computing the potential in the double layer. An approximate solution to Poisson's equation is obtained using a perturbation technique. The expansion is valid for small values of a parameter involving the rate of reaction and the difference in the reciprocals of the diffusion coefficients of the substrate and product. When the diffusion coefficients are identical, the potential reduces to the equilibrium potential for planar geometry. In general, deviations from the Gouy potential arise which ought to be included in calculating apparent reaction rate constants. The deviations become increasingly important at high reaction rates and for large differences in substrate and product diffusion coefficients.

W-POS-A18 DEPENDENCE OF ^{45}Ca EFFLUX ON INTERNAL CONCENTRATION OF CaEGTA IN DIALYZED BARNACLE MUSCLE FIBERS. S. G. Spangler* and F. J. Brinley, Jr., Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Single barnacle muscle fibers were internally dialyzed with solutions containing various concentrations of CaEGTA, and ^{45}Ca efflux was measured. The usual composition of the internal solution (dialysate) was (mM): K:112; Na:14; Cl:29; aspartate:79; Glucose 270; glycine 270; BES:48; pH 7.0. Ca EGTA was added to the dialysate over a range of 20 μM to 10mM, but was always present with an equal amount of free EGTA so that the concentration of free, ionized Ca was nearly constant (nominally 0.2 μM). The external solution was K:10; Na:460; Ca:0; Mg:32; Cl:534; EDTA:0.1; TES:5; pH 7.6. About 2½ - 3½ hours were required to reach a steady state efflux when internal CaEGTA was 2 mM or higher. Longer periods were required at lower concentrations. ^{45}Ca efflux was approximately proportional to internal CaEGTA concentration, about 1 pmole/cm^{2}sec per mM of internal CaEGTA. To estimate how much of this efflux might be leak of undissociated $^{45}CaEGTA$, ^{14}C-EDTA efflux was determined at various concentrations of internal EDTA, the EDTA, being present either in its unchelated form or with various fractions bound to Ca or Mg. ^{14}C-EDTA efflux did not appear to depend on whether it was free or chelated, and was only 7-15% of the ^{45}Ca efflux. Other experiments showed that ^{45}Ca efflux was about the same (or maybe slightly higher) when $^{45}CaEDTA$ and free EDTA were used in place of ^{45}Ca EGTA and free EGTA. We infer from these results that most of the ^{45}Ca efflux was not due to leak of $^{45}CaEGTA$. Analysis of the fibers for Ca content at the end of the experiment showed that an average of 0.43 mmole/kg fiber (wet weight) of endogenous fiber Ca did not exchange with the ^{45}Ca of the dialysate. Supported by NIH Grant NS 08336.

W-POS-A19 INCORPORATION OF DOPAMINE-β HYDROXYLASE INTO A LIPID BILAYER MEMBRANE. Robert Blumenthal and Harvey B. Pollard; Lab. of Theoretical Biology, NCI, and Reproduction Research Branch, NICHD, NIH, Bethesda, MD 20014.

Dopamine-β hydroxylase (DBH) is a major glycoprotein component of chromaffin granule membranes from the adrenal medulla. DBH was isolated from granules by hypotonic shock and purified by affinity chromatography on sepharose-concanavalin A. Purified DBH at a concentration of 0.4 μgr/ml in 0.1 M KCl, pH=7, was found to induce conductance increases in oxidized cholesterol bilayer membranes. The conductance -DBH concentration curve was linear on a logarithmic scale with a slope of one. The current-voltage curve showed a bend towards the voltage axis at V greater than ± 50 mV. There was a threshold in the conductance - KCl concentration curve, with a sharp conductance increase at a KCl concentration larger than 20 mM. This appeared to be an ionic strength effect. From the diffusion potential measurement yielding a slope of -40 mV/log. decade concentration ratio of KCl, a transference number for K^+ of 0.8 was calculated. At low conductance levels discrete current fluctuations at constant voltage were observed with a conductance of 0.6 nmho. These data suggest that DBH may be linked with an ion conductance generating process in vivo.

W-POS-A20 INTERACTION OF PHOSPHOLIPID VESICLES WITH CULTURED, MAMMALIAN CELLS: EVIDENCE FOR VESICLE-CELL LIPID EXCHANGE. R. E. Pagano and L. Huang, Dept. of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Baltimore, Maryland 21210

The uptake of small (~500Å), unilamellar phospholipid vesicles by cultured mammalian cells has been reported by a number of laboratories. Using water soluble markers sequestered within the vesicle, it has been shown that the trapped marker becomes cell-associated upon vesicle treatment, suggesting that a substantial fraction of the lipid uptake by vesicle-treated cells results from a vesicle-cell fusion mechanism. We report here evidence for vesicle-cell lipid exchange. Monolayer cultures of Chinese hamster V79 cells were grown on ^3H precursors (^3H-palmitic acid or 2-^3H-glycerol) for cell lipids. Following a suitable growth period, the cultures were washed in a balanced salt solution (BSS) and incubated for 1 hr at 37°C with unilamellar, dioleyl-1-^{14}C-lecithin vesicles (1 mg lipid/ml BSS). The vesicles were then harvested from the cells and applied to a Sepharose 4B solumn. Comparison of the elution profiles of the vesicles before and after incubation with cells revealed that some of the ^3H cell lipids co-chromatographed with the unilamellar vesicle fractions. These chromatographed vesicles were then extracted with 2:1 $CHCl_3$/MeOH, and the extracted lipids examined by two-dimensional thin layer chromatography. I_2 staining of the chromatograms demonstrated a variety of cell lipids were present in the lecithin vesicles following the vesicle-cell incubation. Radioactive analysis of the developed chromatograms also revealed the presence of ^{14}C in lipids other than phosphatidyl choline. These results demonstrate the existence of vesicle-cell lipid exchange as one pathway in the overall scheme of vesicle-cell interactions. Supported by Carnegie Institution of Washington.

W-POS-A21 INTERACTION OF PHOSPHOLIPID VESICLES WITH CULTURED, MAMMALIAN CELLS: AN EM AUTORADIOGRAPHIC AND CELL FRACTIONATION STUDY. L. Huang and R. E. Pagano, Dept. of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Baltimore, Maryland 21210.

The modification of mammalian cell behavior by incubation with lipid vesicles has been reported by several laboratories. We have found [Nature, 252 (1974) 166] that a substantial amount of exogenous phospholipid is readily taken up by cultured mammalian cells upon incubation with unilamellar lipid vesicles comprised of purified phosphatidyl cholines. In this report, the fate of foreign lipids taken up by cells upon vesicle treatment was determined by cell fractionation methods and high resolution EM autoradiography. In cells treated with dioleyl-9, 10-^{3}H-lecithin vesicles, roughly 20% of the exogenous lipid taken up by the cells was present in the plasma membrane fraction, while the remaining 80% was distributed into intracellular fractions, accompanied by an appreciable amount of metabolism. The foreign lipid present in the membrane fraction represented about 6% of the total plasma membrane lipids, and unlike the internalized lipid, was not degraded. These findings are consistent with our autoradiographic study using lecithin vesicles containing trace amounts of ^{3}H-distearoyl lecithin. Vesicle-treated cells (37°C, 5 or 60 min.) showed the majority of radiolabel in the cytoplasm and possibly accumulated over lipid droplets. Relatively little label was found associated with the plasma membrane or within the nucleus. Cells pretreated with glutaraldehyde (GA) prior to incubation with vesicles showed a similar distribution of radiolabel. The rapid internalization, combined with the ineffectiveness of GA fixation on this process is discussed in terms of a possible continuity of the plasma membrane with cytoplasmic membranes. Supported by Carnegie Institution of Washington.

W-POS-A22 ALTERED GLUCOSE TRANSPORT AND METABOLISM IN CULTURED HUMAN CELLS DEPRIVED OF GLUCOSE. Donald W. Salter* and John S. Cook, Univ. Tenn.-Oak Ridge Grad. Schl. Biomed. Sci. and Carcinogenesis Program, Biology Div., ORNL, Oak Ridge, Tennessee 37830

We have assessed uptake of ^{3}H-glucose into nontransformed human cells by exposing the cells to brief (1-15 min) pulses of isotopic sugar into sparse (log growth), subconfluent, and confluent (contact inhibited) cultures of the diploid HSWP strain derived from human skin. During pulses longer than 1 min ∿70% of the isotope is excreted into the medium as lactate (+ pyruvate). Lactate production and excretion is not affected by cytochalasin B. As reported for other cell types (chick, HeLa; Martineau et al, P.N.A.S. 69, 3407, 1972; Shaw and Amos, B.B.R.C. 53, 357, 1973) 24 hr starvation enhances apparent uptake of radioactivity 10-fold or more. This increase is accounted for by 2-3 fold enhancement of true transport plus a retention of >90% of the radioactivity i.e. little lactate (+ pyruvate) is excreted. The presence of pyruvate or galactose during starvation profoundly alters the distribution of metabolites in a post-starvation assay of ^{3}H-glucose uptake, but in both cases we observe the 2-3 fold enhancement of transport. The enhancement is also observed in the presence of insulin or deoxyglucose. None of these four compounds prevent the starvation effect on transport *per se*. True transport of 3-0-methyl glucose, deoxyglucose, and galactose are also 2-3 fold enhanced in starvation. Two hours after restoration of 5 mM glucose to starved cells, their metabolism is back to normal in the sense that ∿70% of the glucose taken up in a pulse label is being excreted as lactate (+ pyruvate), but transport is still twice normal. These appear to be independently regulated parameters. (Research supported by Natl Cancer Institute and by U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

W-POS-A23 A MODEL SYSTEM APPROACH TO FREEZING INJURY USING LOBSTER SARCOPLASMIC RETICULUM. J. R. Lepock*, P. D. Morse, II*, J. Kruuv*, and A. D. Keith, Intr. by R. S. Morgan, Department of Biophysics, The Pennsylvania State University, University Park, Pennsylvania 16802.

Lobster sarcoplasmic reticulum vesicles (SRV) are capable of taking up calcium by an energy-dependent process which is coupled to ATP hydrolysis. In fresh preparations 1.9 Ca++ atoms are taken up for each ATP molecule hydrolysed. In damaged vesicles uncoupling occurs between Ca++ uptake and ATP hydrolysis and the ratio of Ca++ to ATP is reduced. Thus membrane and protein integrity can be measured. The lobster SRV were frozen under various conditions which included the presence of NaCl in various concentrations and the presence of several cryoprotectants, and the ATPase rate and the amount of uncoupling measured. Freezing rates from 3°C/min to 450°C/min were used. Freezing always stimulated the ATPase rate and increased the amount of uncoupling. Uncoupling was greatest at low and high freezing rates and reached a minimum at 50°C/min indicating two different mechanisms of freezing damage. High concentrations of NaCl during freezing increased the ATPase rate and greatly increased the amount of uncoupling, but had no effect when the SRV were not frozen. ATPase rate and uncoupling increased with increasing salt concentration and reached a maximum around 0.25 M NaCl. Freezing in 5% DMSO at both low and high salt concentrations afforded complete protection from uncoupling. BHT at 1 mM greatly increased the uncoupling due to freezing, but had no effect in the absence of freezing. Other compounds are being checked as cryoprotectants and this appears to be a good system for rapidly assessing the effects of suspected cryoprotectants. In order to deduce more about the molecular basis of freezing damage, ESR studies are being conducted with various spin labels to measure physical changes in membrane lipids as a consequence of freezing.

W-POS-A24 VISCOSITY PROFILE OF SARCOPLASMIC RETICULAR VESICLES. A SPIN LABEL STUDY. Philip D. Morse,II*, Merilee Ruhlig*, Wallace Snipes and Alec D. Keith, Department of Biophysics, The Pennsylvania State University, University Park, Pennsylvania 16802.

Sarcoplasmic reticular vesicles (SRV) were prepared from both lobster and rabbit. A variety of spin labels were used to probe the aqueous region (TEMPONE, TEMPAMINE, myo-inositol pyrrolidine), hydrocarbon region (2N14, 7N14, 3NA, 4NP), and the membrane-aqueous interface (iodoacetamide-TEMPONE, para-amino benzoic acid TEMPONE). Nickel was used to selectively subtract signals originating from the external aqueous zone, external hydrocarbon zone, and the external interfacial zone. Spin label motion was measured by the parameter R_1 ($R_1=W_l (h_l/h_{-1})^2-1$) which was shown to be proportional to bulk viscosity. All R_1 values were normalized with respect to water or oleic acid. Spin labels in the internal aqueous compartment of the SRV were found to rotate 30-50 times more slowly than in the external aqueous environment. Similar results were found for spin labels on the internal and external membrane interface. Hydrocarbon spin labels showed symmetry through the non-polar region of the membrane. TEMPONE was found to be concentrated by a factor of 6 and TEMPAMINE was found to be concentrated by a factor of 3 within the SRV. This is probably due to the known affinity of these spin labels for membrane surfaces. From these data, a viscosity profile was constructed. The major features of the profile show that viscosity increases by a factor of 4 when moving from the external aqueous compartment to the hydrocarbon region, but increases by another factor of 10 when moving into the internal aqueous compartment. These results suggest that one property of cell membranes may be to order water within the cell. We know of no known mechanism for this ordering process.

W-POS-A25 ANOMALOUS BINDING OF THE FLUORESCENT PROBE, ANS, TO PHOSPHOLIPID VESICLES AT THE PHASE TRANSITION TEMPERATURE. K. Jacobson and D. Papahadjopoulos, Departments of Experimental Biology and Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14203.

Permeability studies with lipid vesicles at different temperatures have shown an anomalous local maximum of passive cation transport across phospholipid bilayers undergoing the gel to liquid crystalline phase transition (Papahadjopoulos, et al, B.B.A., 311: 330). In addition, fluorescence intensity of ANS bound to bilayers also shows a maximum at the phase transition temperature, T_c (Sackmann and Träuble, J.A.C.S. 94: 4482). In order to obtain further information on the properties of lipid bilayers undergoing a phase transition, we measured the binding of ANS to sonicated dipalmitoyl phosphatidylcholine (DPPC) vesicles at three temperatures, 25°, 41° (T_c) and 53°, using a conventional fluorimetric assay. Scatchard plots describing the dye binding are biphasic, indicating two binding sites: One class of sites is fewer in number (about one site per 50 to 100 phospholipids), but has a stronger affinity constant; the second class of sites is greater in number, with a much smaller affinity. The binding constant (K) for the high affinity site shows the following temperature dependence: $K(25^{\circ})=0.093\mu M^{-1}$, $K(T_c=41^{\circ})=0.41\mu M^{-1}$ and $K(53^{\circ})=0.23\mu M^{-1}$. Furthermore, the total amount of ANS bound to DPPC shows a maximum at 41°, a result which preliminary equilibrum dialysis confirms. The relation between the enhanced bilayer dye binding at the transition temperature and fluctuation phenomena typical of critical points will be discussed.

W-POS-A26 REPAIR OF DNA STRAND BREAKAGE IN FIBROBLASTS FROM FOUR ATAXIA TELANGIECTASIA (Louis Bar Syndrome) PATIENTS. R.A. Vincent, Jr.*, R.B. Sheridan III*, and P.C. Huang, Dept. Biochemical and Biophysical Sciences, The Johns Hopkins University School of Hygiene & Public Health, Baltimore, Maryland 21205

Ataxia telangiectasia (AT) is an autosomal recessive disease associated with neurological degeneration, immunological defects, and predisposition to malignancy. Fibroblasts from patients with this disease exhibit both increased spontaneous chromosomal breakage and chromatid aberrations following exposure to X-rays in the G2 phase of the cell cycle. These observations suggest a deficiency in the repair of strand breaks in AT cells. In this study, sedimentation profiles of DNA from early passage fibroblasts cultured on cover slips were examined for repair deficiency. The cells were exposed to 10 KR of X-irradiation, allowed to undergo repair for specific time periods, lysed in situ, and centrifuged on linear alkaline sucrose gradients. The resultant sedimentation profiles and the kinetics of repair of DNA in cells from all four patients were essentially the same as those of normal fibroblasts from an unaffected donor. Thus, the increased number of chromosomal aberrations in X-irradiated AT fibroblasts may not be the consequence of a deficiency in the enzymatic repair of strand breaks in DNA. Supported by N.I.H. postdoctoral fellowship GM57140 (R.A.V.), a Kunitz-Worthington Scholarship (R.B.S.), and the National Foundation/March of Dimes (Grant CRBS 300).

W-POS-A27 RADIATION INACTIVATION OF MAMMALIAN CELLS BY SINGLE- AND DOUBLE-EVENTS. J. D. Chapman, A. P. Reuvers*, and C. J. Gillespie, Medical Biophysics Branch, Whiteshell Nuclear Research Establishment, Atomic Energy of Canada Ltd., Pinawa, Manitoba, CANADA.

A linear-quadratic relationship has been used to best describe the radiation inactivation of asynchronous and synchronized populations of Chinese hamster cells for various conditions of chemical modification. A relationship of this form has been proposed on purely empirical grounds by some and to account for microdosimetric and/or target-structure considerations by others. The linear or single-event rate constant was found to have only two values for synchronously growing cell populations whose survival data had been corrected for genome multiplicity; a value of $\sim 6.5 \times 10^{-3}$ rad^{-1} for cells in mitosis and a value of $\sim 2.0 \times 10^{-3}$ rad^{-1} for cells in interphase. The quadratic or double-event rate constant decreased during the cell cycle from a value of $\sim 3.5 \times 10^{-6}$ rad^{-2} for cells in early G_1 to a value of $\sim 0.5 \times 10^{-6}$ rad^{-2} for cells in late G_2 and mitosis. Studies with homogeneous mitotic or G_1-phase cell populations and scavengers of OH indicated that indirect effect contributes to cell inactivation by both single- and double-events. Higher concentrations of OH scavengers were required to protect cells against inactivation by single-events than by double-events. The effectiveness of other chemical radiosensitizers and radioprotectors in modifying cell inactivation is also different for single- and double-events. These studies will be discussed in relation to our current understanding of the radiation chemical mechanisms of these modifiers and the cellular targets.

W-POS-B1 CARBONYL CYANIDE-P-TRIFLUOROMETHOXY-PHENYL-HYDRAZONE (FCCP) INDUCED INCREASE IN THE MICROVISCOSITY AND ALTERATION IN THE ORDER-DISORDER TRANSITION OF THE E. COLI CELL ENVELOPE. S.L. Helgerson* and W.A. Cramer, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

The uncoupler of oxidative phosphorylation FCCP causes an increase in the fluorescence intensity, polarization and lifetime of the cell envelope bound probes N-phenyl-1-naphthylamine (PhNap) and 8-anilino-1-naphthalene-sulfonate (ANS). Increases in these parameters are observed at FCCP concentrations as low as 0.5 μM and are maximal at 3 to 6 μM depending on the cell strain. Changing from an H_2O- to a D_2O- buffering medium increases bound ANS fluorescence two fold but has no effect on bound PhNap fluorescence, indicating that ANS is localized in solvent accessible regions while PhNap is in more hydrophobic regions of the envelope. The rotational relaxation time of each probe calculated from the Perrin equation increases with increasing FCCP concentration, indicating a widespread increase in the microviscosity of the cell envelope. This increase occurs in E. coli ML 308-225, B/1,5, AN180, and AN120 unc A (a mutant lacking a functional ATPase). FCCP changes the temperature dependent order-disorder transition sensed by PhNap in ML308-225 membrane vesicles and B/1,5 whole cells (grown at 20°) to a biphasic linear profile with a break at the transition midpoint temperature.

W-POS-B2 A RAPID METHOD FOR DETERMINING SOLUTIONS AND THEIR STABILITY IN STEADY STATE REACTION DIFFUSION. Barry Bunow and Clark K. Colton*. Department of Chemical Engineering, MIT. Cambridge, Mass. 02139.

Compartmentalized enzyme kinetics in the steady state are modelled by systems of algebraic equations. One is typically interested in the variation of solutions with some continuously varying parameter (p), such as external concentration of substrates or regulators, Thiele modulus, or a Biot number. Formally, we have a set of equations $F_i(p,c_1,c_2,\ldots,c_n) = 0$ which express material balance for all species in all compartments. To solve many non-linear equations is generally difficult by available algorithms when a large number of solutions is required. Here we discuss an alternative approach which provides solutions to these equations and at the same time an evaluation of their stability. The locus of solutions lies on a smooth curve in the space $\underline{X} = (p,c_1,c_2,\ldots,c_n)$. This curve is a solution of the system of differential equations $d\underline{X}/ds = \nabla F_1 \times \nabla F_2 \ldots \times \nabla F_n$. The independent variable in the equation may be taken to be arc length. Stability of the solutions may be obtained as the calculation proceeds, because the first component of the extended cross product is the determinant of the Jacobian matrix of the original system of equations. This matrix must be negative definite in order for the point of the curve to be stable. The initial condition for the differential equation may be found from known limiting physical properties, such as the vanishing of reaction rate at zero substrate concentration, or by obtaining a single solution to the algebraic equations by conventional iterative methods. This procedure has been applied to the problem of determination of multiple solutions to substrate inhibition kinetics in a linear array of cells.

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W-POS-B3 MOLECULAR ORGANIZATION OF THE ERYTHROCYTE MEMBRANE. H.H. Edwards, E. Keyhani, and M. Morrison, Laboratory of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tenn. 38101.

Triton extraction causes human erythrocyte stroma to fragment into 75 to 200 nm diameter vesicles which freeze fracture reveals to be smooth, without the usual intramembranous particles found in unextracted stroma. Thin section of this material also shows numerous vesicles and membrane fragments which are sometimes connected by a fibrillar material. The thickness of the membranes forming the vesicles is estimated to be 6 nm. When the stroma is fixed with 0.02% glutaraldehyde at 24°C and then extracted with Triton X-100, the membrane retains an essentially sheet-like structure which negative staining reveals to be perforated with 30 to 200 nm holes. Transverse thin section reveals a zigzag membrane with numerous gaps of up to 200 nm. Tangential sections confirm the fenestrated appearance of the membrane evident in negatively stained material. The Triton extracted membranes have a filamentous appearance as compared to the more globular nature of normal membrane proteins. Freeze fracture of the fixed and extracted stroma show a membranous network of relatively smooth vesicles with few intramembranous particles. In order to correlate these morphological observations with the molecular organization of the membrane, the concentration of membrane proteins and lipids extracted under the various conditions were analyzed. (This work was supported in part by a grant from NIH, CA 13534.)

W-POS-B4 THE TRANSFER OF BIOLOGICAL PROPERTIES OF PHYCOMYCES BLAKSLEEANUS BY ISOLATED MITOCHONDRIA. Sol H. Goodgal, Marc Kahn* and Jacqueline Keyhani*. Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174.

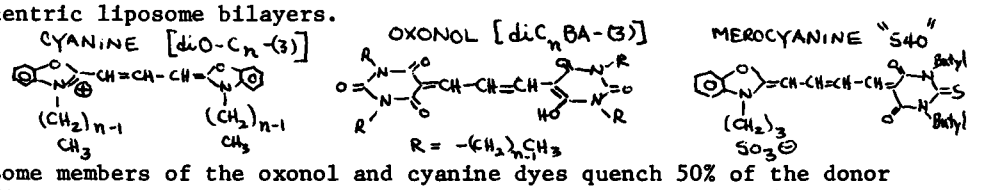
Studies on the hereditary properties of mitochondria are well established in yeast but suffer from an inherent inability to freely manipulate mitochondria outside the cell. We have attempted to obtain a system in Phycomyces in which mitochondria could be used to directly transfer genetic functions. Mutants with increased kanamycin resistance, were isolated and presumed to result from changes in mitochondrial DNA. These mutants served as the source of mitochondria to convert a recipient sensitive to 75 μg or less of kanamycin (kan^s) to cells resistant to greater than 150 μg/ml of kanamycin (kan^r). Mitochondria were isolated and purified by conventional techniques from germlings (germinated spores) of the kan^r strains and added to the cut surfaces of sphorangiophores of the kan^s strain. Spores were isolated from the matured sporangia of sporangiophores regenerated from these cut surfaces and tested for sensitivity to kanamycin. Control spores showed no appreciable difference in resistance to kanamycin, however, greater than 10% of the experimental sporangiophores contained spores that were kan^r.

W-POS-B5 MULTI-FREQUENCY ELECTRICAL IMPEDANCE MEASUREMENTS OF TRIETHYLTIN SULFATE (TET-SO_4)-INDUCED BRAIN EDEMA. W. W. Shelton and G. H. Collins*, Department of Pathology, Upstate Medical Center, Syracuse, New York 13210 and A. H. Nevis, Department of Electrical Engineering and Physics, University of Florida, Gainesville, Florida 32611.

Low frequency electrical impedance measurements (chronic implant) of cerebral brain tissue reflects the available extracellular space (ECS). TET, in chronic dosages, has been shown through electron microscopic and histochemical studies to produce a unique and highly responsive brain edema affecting predominantly the white tissue. The edema grows in severity as the TET-treatment continues and ultimately leads to death. It is characterized by the incorporation of water and electrolytes into interlaminar locations within the myelin with resultant bleb formation. As the blebs of "stagnant" fluid develop, the myelin boundaries protrude into, and thereby reduce, the available functional ECS. Electrical resistance measurements during intoxication in experimental animals revealed progressive, almost linear elevations above baseline values and reached values as great as 40% above baseline during terminal stages of treatment. Dosage levels of 2 mg TET-SO_4 per kg body weight daily in rats produced impedance and neurologic patterns similar to those for rats poisoned with one-half the latter dosage, but in approximately one-half the time. Externally measured brain impedance under the former experimental conditions and expressed as a ratio of low frequency (10 KHz) resistance to high frequency (20 MHz) resistance yielded progressively higher ratios during the study period, again confirming the decrease in available ECS.

W-POS-B6 RATE OF MEMBRANE PENETRATION OF POTENTIAL-SENSITIVE DYES. A.S. Waggoner, D. Sirkin*, R. Tolles* and C.-H. Wang*, Department of Chemistry, Amherst College, Amherst, Ma. 01002

The rates of penetration of members of three classes of fluorescent membrane potential probes into concentric bilayer liposomes were measured. The rates of penetration are inferred from the rates at which each probe, when added to the liposome suspension, quenches the fluorescence of a neutral lipophilic energy donor that is incorporated in the concentric liposome bilayers.



Some members of the oxonol and cyanine dyes quench 50% of the donor fluorescence of unsonicated egg phosphatidyl choline vesicles in less than 10 seconds at 20°C. The fastest quenchers of the diO-C_n-(3) series are the n=5 and 6 derivatives. When $n \gtrsim 10$ the quenching requires hours.

If the vesicles are made up in KCl and suspended in NaCl, the positively charged cyanines are taken up by the liposomes faster when valinomycin is added, judging by the rapid decrease in donor fluorescence. However, the negatively charged oxonol dyes are driven out of the liposomes by the addition of valinomycin to the KCl vesicles suspended in NaCl. The time required for merocyanine 540 and ANS to penetrate the egg phosphatidyl choline liposomes is much slower than that of the fastest cyanine and oxonol dyes. The dye penetration rates will be discussed in terms of lipid solubility and delocalization of dye charge.

W-POS-B7 LIGAND REGULATION OF Na^+,K^+-ATPase (E)-OUABAIN (H^3O) COMPLEXES. J.C. Allen and A. Schwartz, Department of Cell Biophysics, Baylor College of Medicine, Houston, Texas 77025.

E-H^3O complexes can be produced by a number of ligand binding conditions, each achieving the same maximum binding level: I. ATP+Mg+Na; II. ATP+Mg+Na+K; III. ATP+Mg; IV. Mg+P_i. When separated from the binding medium by ultracentrifugation, complexes I and II dissociate quite readily ($t_{1/2}^{I}$=12', $t_{1/2}^{II}$=13') while complexes III and IV are more stable ($t_{1/2}^{III}$=76', $t_{1/2}^{IV}$=83'). Both Na^+ and K^+ added to the dissociation medium stabilize I and II to the same extent. Na^+ is more effective than K^+ in retarding H^3O association to E with Mg+P_i. If Mg+P_i+Na^+, or Mg+P_i+K binding proceeds to maximum, dissociation is slow. K^+ is more effective in retarding the Mg+P_i supported binding rate than the Mg+ATP supported E-H^3O binding rate. Na stimulates the Mg+ATP binding rate perhaps due to a III → I conversion. K^+ retards the ATP+Mg+Na supported H^3O binding to varying extents, depending upon the Na^+ concentration in the binding medium. If either stable complexes III or IV are formed, they can be partially converted to an easily dissociable (I or II) complex by incubation in the presence of ATP+Na^+. This conversion to a dissociable complex is not affected by CDTA or EDTA. It is prevented by Mg^{++}, and K^+ cannot replace Na^+ in the conversion process. If dissociation is carried out in the presence of ligand binding conditions (i.e., I, II, III, IV) dissociation is slow. Dissociation is also slow if binding reaction is stopped by a high concentration of unlabelled ouabain. These data indicate a specific regulatory role of various ligands influencing ultimate conformation of E. (Supported by USPHS grants: HL05435, HL07906 and Contract NIH71-2493.)

W-POS-B8 UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION: KINETIC STUDIES ON BLACK LIPID MEMBRANES. F. Cohen*, S. Chen*, M. Eisenberg* and S. McLaughlin. (Intr. by H. Levy.) Dept. Physiology & Biophysics, SUNY, Stony Brook, N.Y.

For the substituted benzimidazoles DTFB and TTFB, which are uncouplers of oxidative phosphorylation, the membrane permeant species is a HA_2^- complex formed from the neutral acid HA and its anion A^-. In order to investigate the processes occuring at the membrane-solution interfaces we are studying the kinetics of the conductance change upon application of a voltage pulse. We found that the instantaneous current increased superlinearly with increasing voltage, thereby indicating that the HA_2^- complex crossing the membrane is the rate limiting step. We also observed that the ratio of initial to steady state conductances, expressed in terms of $\alpha = (I_o - I_\infty)/I_\infty$ decreased as the buffer capacity of the aqueous phases was symmetrically increased. In a 1M NaCl solution containing 2.5 10^{-5}M DTFB at pH=pK=7.3, α ranged from $\simeq$ 3.8 when the buffer concentration was 1mM to $\simeq$ 0.5 when the buffer concentration was 100mM (for a 150 mV applied pulse). Under these conditions the relaxation time was of the order of 30 μsec. When the voltage was returned to zero, a transient "bounceback" current (distinct from the capacitive transient) was observed which showed a similar marked dependence on the concentration of buffer in the aqueous phases, larger relaxations being observed for lower buffer concentrations. This "bounceback" current saturated with increasing voltage. As the pH of the aqueous phases was varied symmetrically α increased with increasing pH. Rectification was not observed for either asymmetric concentrations of buffers or asymmetric pH. When similar sets of experiments were performed with TTFB at pH values centered around its pK=5.3, substantially smaller relaxations were observed. These relaxations are distinct from diffusion polarization in the aqueous unstirred layers. Supported by NIH grant NS10485

W-POS-B9 EVIDENCE OF STRUCTURAL CHANGES IN THE MEMBRANE OF ESCHERICHIA COLI UPON DISRUPTION OF ENERGY TRANSDUCTION PROCESSES. D. Nieva-Gomez, J. Konisky* and R. B. Gennis, Depts. of Chemistry and Microbiology, University of Illinois, Urbana, Illinois 61801.

Similar effects have been observed for the fluorescence parameters of N-phenyl-1-napthylamine (NPN) bound to Escherichia coli when the cells are treated either with colicin Ia or with agents known to disrupt the normal function of the electron transport chain or energy transduction. Within a few seconds after addition of either colicin Ia, KCN, sodium azide, amytal or carbonyl cyanide-m-chlorophenylhydrazone to a cell suspension (c.a. 5 x 10^8 cells/ml) containing NPN and no carbon source, the fluorescence intensity of the probe starts increasing, reaching a level several times higher than the original level. The change occurs gradually during a period of about 20 minutes, and it is characterized by a shift to shorter wavelengths of the emission spectrum and an increase in fluorescence polarization. The fluorescence intensity of NPN in a cell suspension in the absence of any of these agents changes only slightly over the same period of time. If instead of an inhibitor a carbon source is added to the cell suspension, the fluorescence of NPN remains at the low level for about 20 minutes, followed by a sudden increase in the fluorescence intensity to approximately the same level reached after treatment with the lethal agents listed above. This effect, which is attributed to oxygen depletion in the suspension, is similar in all respects to the effect produced by the inhibitors except that it is readily reversed by bubbling oxygen or air (but not nitrogen) into the sample. These results suggest that colicin Ia affects the proper function of the electron transport chain; these observations may be relevant to the general question of protein-lipid interactions in the bacterial membrane.

W-POS-B10 THE PHOTOELECTRON QUANTUM YIELDS OF HEMIN, HEMOGLOBIN AND APOHEMOGLOBIN: POSSIBLE APPLICATIONS TO PHOTOELECTRON MICROSCOPY OF HEME PROTEINS IN BIOLOGICAL MEMBRANES. O. Hayes Griffith, Rudy J. Dam* and Keith F. Kongslie*, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon.

The potential use of the photoelectric effect to map biological membranes is discussed. In this approach, photoelectron microscopy, the sample is placed in a vacuum system and subjected to ultraviolet light. If the energy of the light source is sufficiently high, the sample surface can emit electrons (photoionize) as well as fluoresce. The photoelectrons are then accelerated, passed through a series of electron lenses and focused onto a phosphor screen (the image plane). In the current series of experiments, hemoglobin is examined as a model system for intrinsic photoelectron labeling experiments. The absolute photoelectron quantum yields of hemin, hemoglobin and apohemoglobin thin films were measured in the 180-230 nm wavelength region. Hemin exhibits a quantum yield of approximately 6×10^{-4} electrons/incident photon at 180 nm, 9×10^{-5} electrons/incident photon at 210 nm, and 2×10^{-6} electrons/incident photon at 230 nm. At all wavelengths the hemin curve lies approximately a factor of 20 above that of hemoglobin and two orders of magnitude above that of apohemoglobin. High image contrast is observed between hemin and apohemoglobin in low magnification photoelectron micrographs, suggesting the feasibility of intrinsic labeling studies involving heme proteins. The quantum yield of hemoglobin is discussed in terms of linear contributions from heme groups and protein weighted by their relative surface areas. The fractional surface areas based on the known structure of hemoglobin are consistent with values derived from the quantum yields of hemin and apohemoglobin.

W-POS-B11 DIRECT EVIDENCE FOR PHOSPHOLIPID INTERACTION WITH PROTEINS. Patricia Jost, T.A. Micka* and O.H. Griffith, Institute of Molecular Biology, University of Oregon, Eugene, Oregon.

The association of lipids with hydrophobic proteins surface(s) or hydrophobic clefts in proteins has been inferred previously from the relative immobilization of fatty acid spin labels when associated with cytochrome oxidase and other membrane protein complexes. We have associated a diacylphosphatidyl choline (containing a spin label on one of the two acyl chains) with a soluble protein (bovine serum albumin) as well as with a membrane protein, microsomal cytochrome b_5. In each case, the protein consists of a single polypeptide chain known to bind fatty acids. Similar binding (immobilization) of the phospholipid molecule is observed, and is established and maintained in the presence of competing excess phospholipid. While the binding constants are unknown, it is apparent that phospholipid-protein interactions persist in the presence of competing phospholipid-phospholipid interactions. The data also suggest the possibility of two components, and the lineshapes are representative of those observed with larger protein complexes such as cytochrome oxidase. These spectral characteristics will be discussed.

W-POS-B12 PHOSPHOLIPID MOLECULAR MOTION IN SARCOPLASMIC RETICULUM (SR) MEMBRANE. D. Davis* and G. Inesi, Laboratory of Physiology and Biophysics, University of the Pacific, San Francisco, California 94115.

Proton Nuclear Magnetic Resonance studies at 220 MHz indicate that over a temperature range from 10° to 30°C the hydrocarbon chains as well as the choline methyl groups of SR phospholipids undergo a transformation from a state of restricted anisotropic motion to one of isotropic fluid-like motion. It is estimated that approximately 60–70% of the hydrocarbon protons and 90% of the choline protons undergo this transition. A relationship between these structural thermal effects and membrane functional parameters is suggested by studies on the temperature dependence of transmembrane Ca^{2+} fluxes, exhibiting a different activation energy above as opposed to below 20°C. Previous spin label experiments are consistent with the occurrence of a temperature induced transition in the physical state of the membrane lipids at approximately 20°C. However, it was demonstrated by high angle x-ray diffraction that nearly all the lipids are already in a disordered conformation at temperatures as low as 5°C. The Nuclear Magnetic Resonance studies indicate that, in SR, a significant change in the character of phospholipid molecular motion occurs near and above the end of a broad order–disorder conformational transition of the lipid hydrocarbon chains. The authors are deeply indebted to Prof. Melvin Klein of the University of California Berkeley for permission to use his NMR facility. This work was supported by the NIH (HL 16607), the American Heart Association and the Muscular Dystrophy Association.

W-POS-B13 K^+ TRANSPORT IN PHOSPHOLIPID VESICLES CONTAINING SODIUM + POTASSIUM ATPase. S. Hilden* and L.E. Hokin, Pharmacology Department, University of Wisconsin Medical School, Madison, Wisconsin 53706

If purified NaK ATPase from Squalus acanthias is added to a sonicated egg lecithin and cholate solution and then dialyzed, vesicles are formed which transport Na^+ (Hilden, Rhee, and Hokin, JBC, 1974). When ATP and Mg^{++} are added to the outside of these vesicles, a ouabain-inhibitable Na^+ uptake is observed. In the same vesicles, a ouabain-inhibitable $^{86}Rb^+$ or $^{42}K^+$ efflux is seen in the presence of outside ATP and Mg^{++}. K^+ and Na^+ transport are inhibited by ouabain only when ouabain is present on the side of the membrane opposite the ATP molecule, i.e., when ouabain is present inside the vesicles. ATP is most effective as a substrate, UTP is least effective, and CTP has intermediate activity both for NaK ATPase and Na^+ and K^+ transport. Na^+ is necessary for the ATP-stimulated K^+ efflux. Experiments with valinomycin show that vesicles trap but don't bind K^+. The Na^+/ATP ratio is 1.42, while the K^+/ATP ratio is 1.04. The Na^+/K^+ ratio is 2.84/2, which compares well with a value of 3/2 in red blood cells. K^+-K^+ and Na^+-Na^+ exchange are also observed in these vesicles.

W-POS-B14 OBSERVATION OF DOMAINS AND PHASE SEPARATION IN HYDRATED PHOSPHOLIPID BILAYER BY ELECTRON MICROSCOPY AND ELECTRON DIFFRACTION. S. Hui and D.F.Parsons, Electron Optics Lab., Roswell Park Memorial Institute,Buffalo, N.Y. 14203 and M. Cowden, Dept. of Nuclear Medicine Services, Veterans Administration Hospital, Buffalo, N.Y. 14215.

Selective area electron diffraction (SAED) and selected reflection dark field electron microscopy (SRDFEM) are applied to study the domain structure and phase separation of hydrated single phospholipid bilayers. SAED shows that domains of several micrometers in diameter are common in single component bilayers, whereas the domains are much smaller in a multi-component system. SRDFEM shows marked contrast between neighboring domains in pure dipalmitoyl lecithin bilayers below the transition temperature. In the case of bilayers formed by mixtures of various mole ratio of cholesterol-dipalmitoyl lecithin, SRDFEM shows ribbon like domains of several thousand Angstroms in width, indicating phase separation between areas of different compositions. Electron diffraction patterns of these bilayers suggest the existance of pure phospholipid domains when the mole ratio of cholesterol-dipalmitoyl lecithin is less than one. Thermally induced phase separation in bilayers of mixtures of phospholipids of different transition temperatures will also be discussed. The results are compared to those from spin label EPR and freeze-fracture experiments. This work is supported by NIH Grant GM 16454 and NCI Grant CA 15330.

W-POS-B15 ASYMMETRY OF THE GLUTAMATE-ASPARTATE EXCHANGE IN MITOCHONDRIA. Marc E. Tischler, Kathryn F. LaNoue*, and James Pachence*. Johnson Res. Fndn., Univ. of Pa., Philadelphia, Pa. 19174

Previous data have shown that the mitochondrial glutamate-aspartate (G-A) exchange, involving the uptake of one H^+ and one glutamate (G) for one aspartate (A) molecule, is electrogenic (LaNoue & Tischler, J. Biol. Chem., in press). Experiments investigating the G-A antiporter symmetry were facilitated by loading rat liver mitochondria with G or A (20-27 nmoles/mg protein). After 2 minutes of incubation in the absence of energy, the exchange fluxes (nmoles/mg protein) measured were 119.2 for G-A, 19.6 for A-G, 14.5 for A-A, and 15.4 for G-G, while in the presence of energy the respective fluxes were 212.8, 9.6, 9.2, and 14.8. This data suggests that the G-A antiporter is asymmetric *in vivo* since flux of the G-A exchange, in the presence of energy, is 14 to 23-fold greater than the other exchanges. In the absence of energy, the asymmetry is still quite striking. Recent pH studies on the G-A exchange showed a stimulation by increasing matrix pH within the physiological pH range. The experimental results support a transport model in which G and A respectively bind to the protonated and deprotonated carrier (experimental pK = 7.9) as monovalent anions; G being transported inward as a neutral molecule and A being transported outward as a negatively charged species aided by an internally negative membrane potential. Inward A movement would necessarily be against the potential gradient, thus accounting for the inhibitory effect of energy on A influx in exchange for G or A, as demonstrated above. G, on the other hand, being transported as a neutral species is unaffected by energy. pH profiles for the G-G and A-A exchanges will be investigated to further establish the asymmetry of the antiporter. (Supported by grants AHA-72-739 and NIH-HL-14461.)

W-POS-B16 OSMOTIC PRESSURE GRADIENTS IN MITOCHONDRIA. J. J. Lemasters*, Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Md. 21205. Intr. by A. L. Lehninger.

Consideration of the permeability characteristics of mitochondrial membranes leads to the conclusion that significant osmotic pressure gradients can exist across the mitochondrial outer membrane. The outer membrane is permeable to low molecular weight solutes. If the oncotic pressure of protein in the outer compartment is unmatched by oncotic pressure externally, then there exists a net osmotic pressure gradient across the outer membrane. Its magnitude is equal to the difference between the outer compartment and external oncotic pressures. No osmotic pressure gradient exists across the inner membrane because of this membrane's ability to unfold in response to a pressure differential. The osmotic pressure gradient across the outer membrane is greatest when mitochondria are in the orthodox configuration and least in the condensed configuration. Based upon adenylate kinase (molecular weight = 21,500) as the major outer compartment protein, it is estimated that the osmotic pressure gradient is 20 mOs or greater when mitochondria are in an orthodox configuration. It is suggested that these osmotic and oncotic pressure gradients underlie the morphological changes observed in rat liver mitochondria during *in vitro* isolation and during changes in metabolic state. Since it is proposed that mitochondria are able to maintain a hyperosmotic internal milieu, the outer membrane would appear to have a functional role of preventing osmotic swelling and lysis.

W-POS-B17 MODIFICATION AND MEASUREMENT OF FORCES BETWEEN LECITHIN LAMELLAE IN WATER. D. LeNeveu and R. P. Rand, Brock University, Canada; V. A. Parsegian, National Institutes of Health, Bethesda, Maryland; D. Gingell, Middlesex Hospital, United Kingdom.

Lecithin immersed in a large amount of water will swell to form a lamellar phase, alternating layers of water (30A) and lipid (40A), in equilibrium with the remaining pure water. Implicit in this stable multi-layer of lipid membranes is a balance of long-range attractive and repulsive forces. Atttraction is probably by van der Waals electrodynamic forces and due to the different polarizabilities of lipid and water. Repulsion is probably electrostatic, either directly between lipid polar charges or indirectly by their competitive interaction for water. Sugar solutes able to go between bilayers will change aqueous polarizability and electrodynamic attraction. We have observed a successive increase and decrease in spacing with progressive addition of sucrose and glucose, consonant with predicted weakening then strengthening of van der Waals attraction. The bilayer structure appears unaffected during this treatment. Also about 12 water molecules for each lecithin appear to exclude small sugars. Dextran, completely unable to penetrate the multilayer, exerts osmotic pressure on the array to decrease lipid spacing. Concurrent measurement of the osmotic pressure and spacing give a repulsive force vs. distance curve; this is in effect a direct measure of the chemical potential energy of water near the lamellar interface. Repulsion is mechanically strong, reaching 2 atmospheres even at 20 A lamellar separation.

W-POS-B18 SOME SIMPLE ALGORITHMS FOR THE PDP-12 AND THE EAI MINIAC COMPUTERS TO USE IN MEASUREMENTS OF CELL PERMEABILITY BY OSMOTIC METHODS. H.G. Hempling, Department of Physiology, Medical University of South Carolina, Charleston, S.C. 29401.

The analysis of membrane function of bone marrow cells or of lymphocytes during transformation has been hampered by the limited number of cells available and by the heterogeneity of populations. Populations with different cell volumes were isolated with a velocity sedimentation cell separator. Simple algorithms were written to interface a particle size analyzer, an EAI Miniac Computer, and a PDP-12 to generate histograms of cell volume. The particle size analyzer and the EAI Miniac were also programmed to record changes in cell volume with half-times as short as one second. The procedure required as few as 50,000 cells/ml and volumes as low as 4 ml. The method was tested on ascites tumors, erythroblastic leukemic cells and lymphocytes. Values for Lp, the hydraulic coefficient, were comparable to those obtained by densimeter methods. Since so few cells are required, a spectrum of permeability measurements is now possible on lymphoid and myeloid cells isolated from 15 ml of peripheral blood or from bone marrow aspirates. The method should make possible large scale screening for the detection of abnormal membrane function. The algorithms will be available to those who wish to use general purpose machines and who want to avoid the needless expense of purchasing special machines to do the same job. (This work was supported by N.I.H. Grant # CA12956-03).

W-POS-B19 ASYMMETRIC EXCHANGE OF VESICLE PHOSPHOLIPIDS BY THE PHOSPHATIDYL CHOLINE EXCHANGE PROTEIN: MEASUREMENT OF FLIP-FLOP RATE. E.A. Dawidowicz* and J.E. Rothman*, Biophysical Laboratory and Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115 (Intr. by M.L. Karnovsky).

The phosphatidyl choline exchange protein (PLEP) was purified from beef liver according to the procedure of Kamp et al. [Biochim. Biophys. Acta 318, 313 (1973)]. This purified protein catalyzes the exchange of phosphatidyl choline (PC) between two membrane systems, for example, phospholipid vesicles and red cell ghosts, as in the present study. The vesicles were prepared from ^{3}H-dioleoyl-PC by sonication followed by sizing on Sepharose 4B. They contained ^{14}C-cholesteryl oleate as a non-exchangeable marker. The kinetics of the exchange (where the ghosts PC is in gross excess) are biphasic. 70% of the vesicle PC is rapidly exchanged whereas the remaining 30% does not exchange during the two hour time course of the experiment. This rapidly exchanged fraction (70%) corresponds closely with the amount of PC on the outer monolayer of these vesicles. The rate of exchange of the remaining 30% of the vesicle PC is determined by the flip-flop rate. Separate experiments indicate that this process occurs with a half-time of several days or longer, a rate substantially slower than that reported by Kornberg and McConnell [Biochem. 10, 1111 (1971)]. Our studies indicate that the PLEP can be used as a tool for determining the distribution of PC between the two sides of a biological membrane. Supported in part by USPHS grants 5 R01 HL14820 and 5 R01 GM19822.

W-POS-B20 VISUALIZING LOCAL FREE IONIZED CALCIUM IN A CELL. B. Rose, Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33152.

A method will be demonstrated for visualizing local Ca^{2+} activity inside a cell. The Ca-sensitive protein aequorin[1] is injected into a cell and the aequorin luminescence, $\sim$ proportional to $[Ca^{++}]^2$, is viewed through a microscope with the aid of a TV camera coupled by fiber optics to an electronic image intensifier. The spatial resolution of the system is 5μ. A film will be shown, illustrating successful applications of the method. Among the phenomena demonstrated will be: the local rise in cytoplasmic $[Ca^{2+}]$ during Ca microinjection into the cell; the Ca^{2+} influx through a point-leak in the cell membrane and the resulting local increase in cytoplasmic $[Ca^{2+}]$; the Ca^{2+} influx through cell membrane and the Ca^{2+} release from intracellular storage sites, mediated by ionophores A2387 or X537A, and the resulting diffuse increase in cytoplasmic $[Ca^{2+}]$; the increase in cytoplasmic $[Ca^{2+}]$ caused by cyanide poisoning; the local changes in cytoplasmic $[Ca^{2+}]$ at a cell junction during junctional uncoupling and recoupling[2].

1 Shimomura, O., and Johnson, F.H. Biochemistry 8, 3991 (1967).
2 Rose, B. and Loewenstein, W.R. Nature (in press).

W-POS-B21 A QUANTITATIVE STUDY OF TUMOR CELL KINETICS AND CHEMOTHERAPY BY THE DISCRETE-TIME KINETIC MODEL. M. Kim*, K.B. Woo, and S. Perry*, Cornell University, Ithaca, New York 14853, and NCI, NIH, Bethesda, Maryland 20014

The growth kinetics of proliferating and non-proliferating cells is studied by dividing the cell cycle into a finite number of parts and by establishing a multidimensional vector. Discrete-time state equations are formulated to describe the cell age distribution during the period of growth and by a linear transformation of the state transition matrices, are employed to predict the time-course behavior of cell size and DNA content distributions. The model is extended to deal with the effects of antitumor drugs on the cell cycle and proliferation kinetics of tumor cell population in which the amount of antitumor drug administered is formulated to be the control function and the transition matrix representing two modes of drug action, namely cell kill and progression delay or accumulation of cells due to drug, is characterized. The drug actions are specified in terms of a fraction of cell killing in each proliferating age compartments and the probability of blocking of a cell at the specific age compartment in the presence of the drug. The model is utilized to examine the effects of cell cycle stage-specific agents such as cytosine arabinoside on spontaneous AKR leukemia. A method of optimal drug schedule is then introduced, which is designed to minimize the cost function that measures the effectiveness of drug schedule as well as penalizes the use of excessive amount of the drug.

W-POS-B22 INHIBITION OF STAPHYLOCOCCUS AUREUS ON A CONTAMINATED ELECTRODE IN THE FEMUR OF THE RABBIT BY LOW ELECTRICAL CURRENT AND ITS RELATION TO STRESS. G. Colmano and S. D. Barranco‡ VPI & SU, Dept. Vet. Sci., Blacksburg, VA 24061, and Montgomery Community Hospital, Blacksburg, VA 24060.

Silver pins, contaminated by half dipping into a broth culture of Staphylococcus aureus (coagulase positive, penicillin resistant), were inserted through the femoro-tibial articulation into the marrow of the femur of both legs of rabbits. While a negative silver lead, used as a ground, was anchored to the subcutaneous tissue of the inguinal region, the current from a resistor on the positive side of a 1.35 V battery, delivering 75 mAH, was connected to the silver pin on the right leg for one hour, so that a calculated output of 6μA (7-8 μA measured between resistor and pin) supplied 2 $\mu A/cm^2$ of silver electrode. After one week the rabbits were euthanatized, the pins were removed and assayed for Staphylococcus aureus. The rabbits were also bled once before surgery, and again before euthanasia. Hematocrit, and blood plasma levels of glucose, ascorbic acid, corticosterone and ceruloplasmin were determined. An increase in ceruloplasmin and corticosterone correlated with a drop in glucose, and a corresponding increase in the refractive index, all pointing to the high level of stress on the rabbits. There were no changes in hematocrit and ascorbic acid. Growth inhibition was indicated by lower or negative plate counts from the right, current treated leg, as compared to the left control leg, while very little or no Staphylococcus aureus could be cultured from the pins from either leg. A high level of stress by itself could explain the bacterial inhibition. This project was supported by Hatch VA-0616188.

W-POS-B23 ELECTRICAL CHARACTERISTICS OF CHICK LEG MUSCLE IN TISSUE CULTURE. D.L. Gruol*(intr. by K. Kusano), Dept. of Biology, Illinois Institute of Technology, Chicago, Illinois 60616.

The electrical characteristics of developing chick leg muscle in tissue culture from 2-44 days have been studied using dispersed cell cultures. Culture conditions were adjusted so that unbranched, cylindrical myotubes ranging from 125-3270μ in length and 6-20μ in diameter were obtained in addition to branched fibers. The cable properties of such unbranched fibers may be determined using the finite cable equation. The off transient analysis was used. The average Vm and surface area (SA) increased with age up to 21 days in culture. Considerable variation in Rm, τ and Cm was observed, however, these data could be separated into two groups based on a comparison of the Rm, τ and Cm for individual fibers. One group of fibers had a relatively high Rm and τ and a relatively low Cm. Representative average values for this group are 2380Ωcm^2, 9.0 msec and 3.7 μF/cm^2. The second group of fibers had a lower Rm and τ but a higher Cm with representative values of 486Ω cm^2, 4.26 msec, and 9.72 μF/cm^2. Steady state analysis was also applied for comparison of Rm values and similar results were obtained. Myotubes with as few as 6 nuclei showed the characteristics typical for each group. There was no significant difference in Vm or SA between the two groups, and morphological differences were not found on the light microscope level. In response to depolarizing pulses or annodal break pulses, myotubes from both groups produced graded action potentials. In smaller numbers where nerve and muscle were cultured together and where neuromuscular activity was seen, both types of fibers were found. The presence of these two groups of fibers might correlate with the fast and slow muscle found *in vivo* in chick leg muscle. (Supported in part by NINDS grant (NS 09618) to Dr. K. Kusano)

W-POS-B24 COMPARATIVE STUDIES ON POLYDEOXYRIBOCYTIDYLATE (dC), POLYRIBOCYTIDYLATE (rC), AND POLY-2'-O-METHYLRIBOCYTIDYLATE (mC) AND POLYDEOXYRIBOINOSINATE (dI), POLYRIBOINOSINATE (rI), AND POLY-2'-O-METHYLRIBOINOSINATE (mI). J.Alderfer, I.Tazawa*, S.Tazawa*, and P. Ts'o. Johns Hopkins Univ., Div. of Biophys., School of Hygiene & Public Health, Baltimore,Md. 21205.

As reported previously (Biophys.Soc.Abs. (1972) 12, 168a), the CD spectra of rC and mC are similar (although not identical), and both are different from dC. The extinction coefficients, ϵ(mM), can be ordered as: mC≈rC<dC. Extensive pmr studies support the base-stacking order obtained from CD and UV data but indicate that each polymer has a different mode of base-stacking. The $J_{H1'-H2'}$ data reveal that the furanose conformation of dC is unrelated to base-stacking and mostly C2'-endo, while the furanose conformation of rC and mC is related to base-stacking, favoring C3'-endo with increased base-stacking (especially mC). The Tm's of the self-complex in acidic solution are ordered: dC>rC>mC. This order can be predicted from the extent of base-stacking of the single strand, as in the case of the poly-adenylate series (Biochem.(1974) 13, 1615). The CD spectra of rI and mI are also similar to each other and both are different from dI; the order of ϵ(mM) is mI, rI>dI. The pmr data supports this order indicating dI base-stacks more than rI and mI. The $J_{H1'-H2'}$ data reveal the furanose conformation of dI (like dC and dA) is mostly C2'-endo and unrelated to the extent of base-stacking, while rI and mI exhibit a slight shift to C3'-endo forms with increased base-stacking. At neutral pH, mI forms a self-complex at substantially lower salt concentration than rI and dI, contrary to the expectation from the order of stacking of the single strands. This can now be partially understood since the data indicates that mI forms a double-stranded complex instead of a triple-stranded complex formed by rI and dI. (Supported by NIH grant GM 16066-6 and NSF grant GB 30725X2.)

W-POS-B25 RADIOPROTECTION OF MICE BY SUPEROXIDE DISMUTASE. A. Petkau and W. S. Chelack, Medical Biophysics Branch, Atomic Energy of Canada Limited, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, Canada.

A single intravenous dose of superoxide dismutase at 35 μg/g body weight significantly protected Swiss white mice from the lethal and haematological effects of 650 rads of X-rays. The number of mice surviving for 30 days (post-irradiation) in the enzyme-treated groups was 3.5 and 3.1 times larger, respectively, than in the control groups which received 0.9% saline or the inactivated enzyme. In the blood, the number of circulating neutrophils was less severely depressed in the enzyme-treated mice than in the others. Furthermore, after the tenth post-irradiation day the number of lymphocytes, neutrophils, platelets, and reticulocytes was always highest in the enzyme-treated group. These results suggested that superoxide dismutase must radioprotect the undifferentiated stem cells and support for this hypothesis was obtained from marrow transplantation experiments which showed that the radiation sensitivity of the viability of bone marrow cells from the enzyme-treated mice was reduced at least by a factor of 2 over that of similar cells from saline injected mice.

W-POS-B26 RADIATION PHYSICS OF THE ACTA-SCANNER. R. S. Ledley, National Biomedical Research Foundation, Georgetown University Medical Center, Washington, D.C. 20007.

The ACTA-Scanner (Automatic Computerized Transverse Axial) is a tomographic x-ray scanner (1) which can scan the cross sections everywhere on the whole body and reconstruct the corresponding images. Each scan starts with a translation scan-pass during which both the x-ray tube and the crystal detector are moved in parallel past the patient's body. As the beam transverses the body, some part of it is absorbed and the remainder is detected. The absorption along any path depends on the sum of the absorption coefficients of the tissues through which the beam passes. The x-ray profile is formed by taking 160 measurements of transmitted x-ray intensity during the scan-pass. The translation carriage is then rotated and another scan-pass is made producing another profile, and so forth. A completed scan can be composed of up to 180 scan-passes, with the carriage being rotated one degree for each pass. Thus, each point has been "measured" 180 times. The 180 x-ray profiles of the section are then processed by the computer which "reconstructs" the tissue absorption coefficient at each point, forming the array of 160 x 160 picture points. Due to the complex nature of the x-ray spectrum of the detecting system and of the reconstruction algorithms, the biophysical interpretation of the results is a formidable analytic problem.

The results of phantom studies and clinical investigation will be presented concerning the meaning and interpretation of the absorption coefficients reconstructed by the ACTA-Scanner.

(1) Ledley, R. S., Science, 186, no. 4160, 207-212, Oct., 1974

W-POS-B27 INHIBITION OF POLYPEPTIDE CHAIN INITIATION IN E. COLI BY ELONGATION FACTOR G: STUDIES ON THE INTERACTION OF FACTOR G WITH RIBOSOMAL SUBUNITS. S. Lee-Huang and H. Lee*, Dept. of Biochemistry, New York Univ. School of Medicine, New York, N.Y. 10016.

We have reported on the isolation of an inhibitor of polypeptide chain initiation in E. coli and have recently identified this inhibitor (PNAS (1974) 71, 2928) as the chain elongation factor G (EF-G). Upon studying the interaction of EF-G with E. coli ribosomal subunits, using sucrose density gradient centrifugation and polyacrylamide-agarose gel electrophoresis, we find that this factor promotes the association of 30S and 50S subunits to 70S ribosomes. In the absence of 50S particles EF-G caused some aggregation of the 30S subunits. The subunit-associating effect of EF-G was not modified by GTP, GMPPCP, fusidic acid, initiator tRNA, or initiation factors. Since inhibition of initiation might involve an interaction of EF-G with the 30S subunit, the possibility of interaction was studied with ^{35}S-labeled EF-G. However, under the conditions employed, no interaction was detected. Experiments are in progress to determine whether glutaraldehyde fixation might enable the detection of a labile complex between ^{35}S EF-G and 30S subunits.

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