W-POS-A1 PREPARATION OF HOMOGENEOUS VESICLES AND MICROEMULSIONS OF EGG PHOSPHATIDYLCHOLINE AND CHOLESTERYL OLEATE. Lucy Shorr*, G.G. Shipley, D.M. Small, and Barry Sears, Biophysics Division, Boston University School of Medicine, Boston, Mass. 02118.

Cholesterol esters are one of the major components in serum lipoproteins and accumulate in the arterial wall during the pathogenesis of atherosclerosis. To investigate the role of cholesterol esters in membranes and serum lipoproteins, we have developed model systems composed of mixtures of cholesterol ester and phospholipid. Homogeneous sonicated egg phosphatidylcholine (PC) vesicles can be prepared that contain up to 4 mole % cholesteryl oleate (CO). These vesicles are homogeneous as characterized by light scattering, trapped volume measurements, and chemical composition. At higher CO contents, microemulsions consisting of a CO core surrounded by a PC monolayer are formed. The microemulsion structure is indicated by the lack of trapped $K_3F3(CN)_6$ within the particle. Using negative stain electron microscopy, microemulsions of 260 Å diameter are formed at a CO/PC ratio of 1.2, and 160 Å particles are formed at CO/PC ratio of 0.5. Theoretical calculations using a surface area of 60 $Å^2$ for PC, a molecular volume of 1100 $Å^3$ for CO, and a PC monolayer thickness of 20 Å, predict that a microemulsion with a CO/PC ratio of 1.2 should have a diameter of 230 Å, whereas a CO/PC ratio of 0.5 give a particle of 150 Å diameter. Fluorescence depolarization studies using diphenyl hexatriene, indicate that the large microemulsions of 260 Å diameter undergo a reversible thermal transition at 30°C, whereas the small microemulsions of 160 Å undergo a reversible thermal transition at 27°C. Both the large and small microemulsions are stable for at least 7 days at either 37°C or 10°C as determined by their elution profile on gel columns. The vesicles and microemulsions provide well defined model systems to study the role of lipid-lipid interactions in both membranes and serum lipoproteins.

W-POS-A2 THE LINKED EFFECTS OF ANESTHETICS AND PRESSURE ON THE THERMOTROPIC BEHAVIOR OF DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES. D.B. Mountcastle,* R.L. Biltonen,* and M.J. Halsey,* Departments of Biochemistry and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia, 22903. Introduced by C.M. Anderson.

The effects of anesthetics and pressure on the gel-liquid crystaline transition of Dipalmitoylphosphatidylcholine (DPL) lipsomes has been investigated using differential scanning calorimetry adapted for use with a pressure cell. Halothane reduced the transition temperature ($T_m$) but the total enthalpy change ($\Delta H$) was not perturbed. The fact that the broadness of the transition increased was most interesting. Pressure increased the $T_m$ as expected from measured volume changes but did not change either the transition width or the $\Delta H$. However, pressure "reversed" the effects of the anesthetic; at any given halothane concentration increasing pressure raised the $T_m$ and caused a sharpening of the transition. Insofar as the transition width, at constant $\Delta H$, is inversely related to the cooperative domain size we suspect that anesthetics may influence biological activity primarily by reducing the extent of thermodynamic linkage within the lipid portion of the membrane and the pressure reversal of the anesthetic effect is the result of increasing the cooperative domain size.

W-POS-A3 ANTIBODY-MEDIATED TARGETING OF LIPID VESICLES: ENHANCED VESICLE BINDING DOES NOT IMPLY INTRACELLULAR DELIVERY OF TRAPPED MATERIAL. J.N. Weinstein (LTB), R. Blumenthal (LTB), S.O. Sharrow* (IB), and P. Henkart* (IB), National Cancer Inst., NIH, Bethesda, MD 20014

If lipid vesicles are to be used for the selective delivery of physiologically active agents to specific cells or tissues, a means of targeting will be necessary. Toward this end, we used antibody to the trinitrophenyl hapten (sheep IgG anti-TNP) as a bridge between vesicles carrying the dinitrophenyl (DNP) hapten and lymphocytes carrying TNP (antibodies to DNP and TNP cross-react).

Small, mostly unilamellar vesicles containing 200 mM 6-carboxyfluorescein (6-CF) were formed by sonication of a mixture of dioleyl lecithin and N-DNP-aminocaproyl phosphatidylethanolamine (95:5 w/w). Free 6-CF was removed by gel filtration. Human peripheral blood lymphocytes were externally TNP-modified with trinitrobenzene sulfonate (viability being maintained). These cells were incubated at 37°C with sheep IgG anti-TNP (0 to 100 μgm/ml) for 15 minutes, and the vesicles were then added for an additional incubation time of 30 minutes at 37°C. The cells were washed and then analyzed by fluorescence microscopy, fluorometry, and flow microfluorometry.

The major findings were as follows: (i) very little (if any) more 6-CF was released from vesicles into the lymphocytes than was the case with controls using non-specific IgG instead of anti-TNP; (ii) however, much greater numbers of vesicles were bound to the cells than was the case with the controls; (iii) high concentrations of anti-TNP and of vesicles resulted in a pattern of fluorescence suggesting aggregation of vesicles on the cell surface.

These studies show the necessity of caution in equating vesicle-cell association with delivery of water-soluble materials to the cell interior.

W-POS-A4 MEMBRANE INTERACTION OF THE C5b-9 COMPLEMENT ATTACK COMPONENTS WITH LIPID BILAYERS. David W. Michaels, Aaron S. Abramovitz*, Carl H. Hammer* and M. M. Mayer*. Depts. of Microbiology and Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore, Md. 21205

We have previously shown that the ion permeability of lecithin black lipid membranes (BLM), as measured by electrical conductance, increases modestly after sequential addition of the complement complex C5b,6 and components C7 + C8. Subsequent addition of C9 greatly amplifies this change (PNAS, U.S.A. 73, 2852-2856, 1976). We now report experiments with BLM prepared from oxidized cholesterol or glycerol monoolein with hexadecane which show that C5b,6 and C9 individually increase membrane permeability in a fashion suggesting the formation of transmembrane channels. In contrast, neither C7 nor C8 produce permeability changes when used alone; however, both factors markedly augment BLM conductance when used together with C5b,6 and C9. Further, C7 and C8 can interact with C5b,6 from both cis and trans side of the bilayer. In addition to revealing the interaction of C5b,6 and C9 with membrane lipid, these experiments show that phospholipid is not required for the action of C5b-9; instead the only requisite is for a hydrophobic milieu of bimolecular dimension. Finally, the greater sensitivity of oxidized cholesterol and monoolein BLM may reflect a dependence of the complement attack mechanism on membrane thickness. (Supported in part by USPHS Grant AL-02566, NSF Grant GB 38628 (M. M. Mayer) and USPHS Grant GM-05919 (A. L. Lehninger)).

W-POS-A5 MECHANISM OF LIPID-SOLUBLE IONS ACROSS MEMBRANES. T. Ree Chay, Department of Life Science, University of Pittsburgh, Pittsubrgh, PA 15260

A flux equation for the lipid-soluble ions across thin membranes has been derived by utilizing Eyring's multi-barrier kinetics. This equation shows that the Nernst-Planck equation is applicable only in limited cases (e.g. when the applied voltage is very low). We have used this equation to the uncoupler transport to study the pH-conductance relation, the current-voltage relation, and etc. Our model describes satisfactory the experimental data of uncoupler transport for the transient as well as steady state cases.

W-POS-A6 DIFFERENTIAL SCANNING CALORIMETRY OF LECITHIN-CHOLESTEROL MIXTURES. S.Mabrey, P.L. Mateo*, and J.M. Sturtevant*,Chemistry Department,Yale University, New Haven, Ct. 06520

The almost universal occurrence of cholesterol in natural membranes suggests its importance as a fundamental component for the structure and function of membranes. Earlier work in this laboratory showed that the transition enthalpy of a pure phospholipid bilayer disappears at a cholesterol content of 33 mole percent (Hinz and Sturtevant, J. Biol. Chem., 247, 3697-3700 (1972)). Since additional complexities for this system have been suggested by others(e.g. Kleemann and McConnell, Biochim. Biophys. Acta, 419,206-222 (1976)), we have undertaken a careful study of synthetic lecithin-cholesterol mixtures using highly sensitive differential scanning calorimetry. After mixing appropriate amounts of lecithin and cholesterol in $CHCl_3$, the solvent was removed and the remaining lipids dispersed in 0.01 M phosphate buffer, pH 7, by vigorous vortexing above the transition temperature of the lecithin. Suspensions (0.2% by weight lecithin) were cooled slowly before filling the calorimeter. As expected, the data indicate that the highly cooperative transition of the pure lecithin undergoes considerable change on the addition of increasing amounts of cholesterol. We have been able to resolve the mean heat absorption in binary mixtures of cholesterol and dimyristoyl-L-α-lecithin(DML) or dipalmitoyl-L-α-lecithin(DPL) into two components, one sharp and one broad. The sharp component, which corresponds to the midpoint of the transition of pure lecithin ($T_m$(DML)=23.5°; $T_m$(DPL)=41.5°), has completely disappeared at a cholesterol content above 30 mole %. The broad transition which is only present in cholesterol-containing suspensions, occurs at a higher temperature than the $T_m$ of the sharp transition and disappears above 50% cholesterol. We are extending our work to the effect of cholesterol upon the phase transition occurring in mixtures of DML and DPL of varying composition to assess whether cholesterol will show a preference for one of the phospholipid species present.

W-POS-A7 UNIFIED PHASE BEHAVIOR OF HYDRATED SYNTHETIC LECITHINS. Martin J. Janiak, Biophysics Division, Boston University School of Medicine, Boston, MA. 02118.

X-ray diffraction and differential scanning calorimetry have been utilized to determine the phase behavior of dimyristoyl lecithin (DML) and dipalmitoyl lecithin (DPL). The structures associated with the thermal pretransition($T_1$) occurring prior to the chain melting transition($T_2$) have already been defined (Janiak et al.,Biochemistry,15:4575,1976). Existing data on dilauroyl lecithin (DLL) and distrearoyl lecithin (DSL) (Ladbrooke & Chapman, Chem. Phys. Lipids,3:304,1969;Tardieu et al.,75:711,1973) together with the results on the phase behavior of DML and DPL suggest unified phase behavior of these synthetic lecithins. At low temperature,($<T_1$), a one dimensional lamellar structure whose hydrocarbon chains are stiff, fully extended but tilted with respect to the plane of lamellae exists ($L_{\beta'}$). At $T_1$, this structure transforms to a two dimensional lattice, consisting of stacked lamellae distorted by a periodic ripple in the plane of the lamellae, the hydrocarbon chains remaining tilted ($P_{\beta'}$). This structure exists at hydration values $n \geq 11$ (n is molecules water per molecule phospholipid). At $n < 11$, the structure $L_{\beta'}$ predominates. Both the structures $L_{\beta'}$ and $P_{\beta'}$ hydrate to a maximum value of $n \simeq 15$, after which water exists as a separate phase. At $T_2$ the structure reverts to one dimensional lamellar, the hydrocarbon chains now assuming a liquid-like conformation ($L_\alpha$). This transition extends over all water contents, excess water coexisting with $L_\alpha$ at $n > 23$ for DLL, $n > 25$ for DML, $n > 27$ for DPL and $n > 29$ for DSL. These lyotropic phase boundaries can be accounted for by: 1)the existence of a hydration shell for lecithin at n=11;2) the weakening of attractive van der Waals forces between bilayers (Le Neveu et al Science 191:339,1975) correlating with the mean molecular areas of these lecithins above or below the chain melting transition.

W-POS-A8 PROTON NMR STUDY OF THE DECAY OF BILAYER COMPOSITIONAL ASYMMETRY GENERATED BY A PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN. J.M. Shaw*, W.C. Hutton*, B.R. Lentz, and T.E. Thompson (Intr. by J.W. Ogilvie) Department of Biochemistry, University of Virginia, Charlottesville, Va. 22901.

Transbilayer compositional asymmetry was generated in single-lamellar vesicles formed from $-N(CD_3)_3$ egg phosphatidylcholine by incubation with erythrocyte ghost membranes in the presence of a purified phosphatidylcholine exchange protein prepared from beef liver. Greater than 80% of the $-N(CD_3)_3$ phosphatidylcholine in the external face of the bilayer vesicles was replaced by $-N(CH_3)_3$ phosphatidyleholine from ghost membranes after a 24 hr incubation at 37°C. Proton NMR studies utilizing $Pr^{+++}$ as a shift reagent showed that 90% of the exchanged $-N(CH_3)_3$ phosphatidylcholine was found on the external face of the vesicle whereas about 10% occurred on the interior face of the vesicle wall. The decay of the transbilayer compositional asymmetry by exchange migration of $-N(CH_3)_3$ and $-N(CD_3)_3$ phosphatidylcholines, as followed by NMR spectroscopy over a 6 day period was shown to be a slow process with a half life of greater than 30 days at 25°C. Appropriate controls established the integrity of the vesicles throughout the 6 day period. The cholesterol content of the erythrocyte ghost membranes used to prepare the asymmetric vesicles was reduced 35-40% by prior incubation with phosphatidylcholine liposomes. During preparation of the asymmetric vesicles, spontaneous cholesterol movement from the 'depleted' ghosts resulted in a final cholesterol content in the asymmetric vesicles of 6-8 mol%. (This work was supported by USPHS grant GM 14628 and postdoctoral fellowship GM05190 to J.M.S.)

W-POS-A9 PHOSPHATIDYLCHOLINE CARBON-13 LABELED CARBONYLS AS A PROBE OF BILAYER STRUCTURE. C.F. Schmidt*, Y. Barenholz*, C. Huang, T.E. Thompson, and R.B. Martin*, Depts. of Biochemistry and Chemistry, University of Virginia, Charlottesville, Va. 22901.

Dipalmitoyl and dihexanoyl phosphatidylcholine have been synthesized using fatty acids which have the acyl carbonyl carbons enriched with carbon-13. The chemical shifts of these carbonyl carbons, which are known to be sensitive to intermolecular interactions, have been measured in a variety of solvents, including aqueous dispersions. The use of dihexanoyl phosphatidylcholine permits the observation of molecules in both monomer and micelle forms in aqueous solutions. Carbon-13-proton two- and three-bond coupling constants have also been measured. From these data, it can be concluded that when the molecules are in bilayers the observed shifts are determined by hydrogen bonding of the carbonyl oxygens with the water, even though there is partial exclusion of water molecules from this region of the bilayer. Furthermore, the relative shifts of the two carbonyl carbon-13's indicate that the fatty acid esterified to the 1-carbon of the glycerol is less accessible to water than that esterified to the 2 carbon of glycerol, and is therefore buried deeper in the bilayer.

This research was supported by USPHS grants GM-14628, HL-17576, and NSF grant TCM-7410484.

W-POS-A10 HEAD GROUP CONFORMATION IN PHOSPHOLIPIDS: A $^{31}P$ NMR STUDY OF ORIENTED DPL BILAYERS. R.G. Griffin*, L. Powers, J. Herzfeld, R. Haberkorn* and P.S. Pershan, Francis Bitter National Magnet Laboratory, M.I.T., Cambridge, Mass. 02139; Division of Engineering and Applied Physics, Harvard University, Cambridge, Mass. 02138; and Biophysical Laboratory, Harvard Medical School, Boston, Mass. 02115.

$^{31}P$ NMR spectra of oriented monodomain dipalmitoyllecithin (DPL) monohydrate bilayers show a doublet structure when the bilayer perpendicular makes an angle of $\sim 50^{o}$ with $H_o$. Using the known orientation of a $^{31}P$ chemical shift tensor, which we have measured, we conclude from these spectra, that the O-P-O plane, where the O's are the nonesterified oxygens of the $PO_4$, is tilted at $\sim 50^{o}$ with respect to the bilayer plane. This orientation of the $PO_4$ places the choline part of the head group in an orientation approximately parallel to the bilayer plane and, furthermore, it will explain the 60ppm $^{31}P$ powder spectrum observed for DPL in excess water. Thus, we believe that this tilted head group conformation is the phospholipid conformation which exists in model and biological membranes.

W-POS-A11 INVESTIGATION OF STRUCTURAL ORGANIZATION OF $CA^{++}$ IN LIPID BILAYERS BY THE EXTENDED X-RAY ABSORPTION FINE STRUCTURE TECHNIQUE. L. Powers, P. Eisenberger*, and J. Stamatoff, Bell Laboratories, Murray Hill, N.J. 07974.

The EXAFS technique, which employs synchrotron radiation as a tunable x-ray source, measures the x-ray absorption of an element versus photon energy. The absorption above threshold is modulated by the photoelectrons emitted by the absorbing atom that are scattered from neighboring atoms causing interference in the amplitude of the initial emitted photoelectron wave.

In the past this technique has been successfully applied to many organometallic complexes of biological origin to determine both the charge of the metal atom and the coordination distance to neighboring atoms. Recently, calcium binding in lipid bilayers has been studied by this technique as a function of temperature and water content. Comparison between binding in monodomain and unoriented lipid-water systems was made and binding of other divalent cations was investigated.

W-POS-A12 AN INTERPRETATION OF THE C-H STRETCHING REGION IN THE VIBRATIONAL RAMAN SPECTRA OF PHOSPHOLIPIDS. M. R. Bunow and I. W. Levin,* Laboratory of Chemical Physics, National Instiutes of Health, Bethesda, Md. 20014.

The 2900 $cm^{-1}$ region in the vibrational Raman spectrum of phospholipids reflects predominantly the C-H stretching modes of lipid acyl chains. Having surveyed a variety of phospholipids, we note that the peak at about 2883 $cm^{-1}$, composed of methylene C-H asymmetric stretching modes, broadens and moves to higher wavenumbers with increasing introduction of disorder into the initially all-trans hydrocarbon chains. Simultaneously, the IR-active band centered at 2925 $cm^{-1}$, containing methylene C-H asymmetric stretching modes, becomes increasingly apparent in the Raman spectrum. The parameter $I_{2935/2880}$ monitors these changes. Using, in part, spectra of $(^2H)_9$-dipalmitoyl lecithin which has a totally deuterated choline group, we argue that the C-H symmetric stretching vibration of the acyl chain terminal methyl group at 2935 $cm^{-1}$ is insensitive to the state of the phospholipid. We demonstrate the utility of the parameter $I_{2935/2880}$, as well as $I_{2935/2850}$ and $I_{2880/2850}$, in describing the effects of the polyene antibiotic amphotericin B on lipid systems containing cholesterol. Changes in the amphotericin B spectra are also discussed. Supported in part by a National Institutes of Health fellowship #1 F32 GM05127-01 to M.R.B.

W-POS-A13 MOLECULAR OXYGEN IN A LIPID BILAYER MEMBRANE. W. Z. Plachy, D. Windrem, and G. Drobny, Chemistry Department, San Francisco State University, San Francisco, California 94132.

The ESR spectral linewidth of a small hydrophobic spin probe, per-deutero di-t-butyl nitroxide, dissolved in a variety of solvents is shown to be very sensitive to dissolved molecular oxygen. When this probe is partitioned between the lipid bilayer phase and the aqueous phase of a phospholipid dispersion in water, well resolved X-band ESR spectra are obtained from both phases. Assuming that the oxygen dependent linewidth is due to a heteroradical spin exchange mechanism, the excess linewidth in each phase can be related to the product of the oxygen concentration and the apparent oxygen diffusion coefficient in that phase using existing theory.

The apparent oxygen diffusion coefficient in di-palmitoyl lecithin bilayers at 45°C is about $1.6 \times 10^{-5}$ $cm^2/sec$ if the partition coefficient is assumed to be about 5 in favor of the bilayer phase. This number is in good agreement with values obtained from fluorescence quenching experiments.

Experimental techniques used include gas permeable ESR sample tubes and computer deconvolution of overlapping signals at high oxygen concentrations.

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W-POS-A14 MEASUREMENT OF REPULSION BETWEEN CHARGED PHOSPHOLIPID BILAYERS. A.C. Cowley, N. Fuller, R.P. Rand, Brock University, St. Catharines, Ontario, Canada L2S 3A1 and V.A. Parsegian, National Institutes of Health, Bethesda, Maryland 20014 USA

We have previously reported the direct measurement of forces between egg lecithin bilayers using an "osmotic stress technique" (LeNeveu, Rand & Parsegian, Nature 259, 601 (1976)). We now apply this same method to measure net repulsive forces between pure phosphatidyl glycerol bilayers as well as egg lecithin bilayers containing various amounts of charged lipids: sodium oleate 3%, 5%, or 10%, sodium phosphatidyl inositol 10%, or sodium phosphatidyl glycerol 5% or 50%. We have been able to plot the force between lipid bilayers as a function of bilayer separation $d_w$ in spite of phase separation in some of the systems. All the force curves are similar in shape and quantitatively consistent. For $d_w$ less than approximately 30 Angstroms the change in force with $d_w$ is rapid and characteristic of hydration forces (op. cit.); for 30 Å $< d_w <$ 120 Å, the rate of change of force with $d_w$ is smaller and its variation with charge density agrees at least qualitatively with theoretical calculations of electrostatic repulsion. Quantitative analysis however suggests that in some cases not all the lipid is ionized. Theoretical analysis also supports the experimental observation that two distinct lamellar phases may sometimes coexist even if the two phases be of similar lipid composition.

W-POS-A15 PARTITION OF A FLUORESCENT PROBE BETWEEN CRYSTALLINE AND LIQUID DOMAINS OF A LIPID BILAYER. M.C. Foster and J. Yguerabide, Dept. of Biology, University of California at San Diego, La Jolla, Ca. 92093

In using fluorescent probes to study the fluidity and the lateral phase separations of biological membranes, it is essential for the interpretation of the measurements to know whether the probe molecule partitions into the crystalline as well as the liquid phases of the membrane. To obtain this information for a water-insoluble fluorescent probe in a two-component lipid bilayer, we have developed and used a simple mathematical analysis which allows us to estimate the partition coefficient from the known phase diagrams and the observed variation of fluorescence intensity with temperature. The results obtained using the fluorescent probe perylene in vesicles composed of dipalmitoyl phosphatidylcholine/ distearoyl phosphatidylcholine mixtures and of dipalmitoyl phosphatidylcholine/ dipalmitoyl phosphatidylethanolamine mixtures are consistent with a partition coefficient of the order of one for the partition of perylene between crystalline and liquid domains.

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W-POS-A16THE EFFECT OF PHASE TRANSITIONS ON LATERAL DIFFUSION IN PHOSPHOLIPID MEMBRANES. E-S Wu[a], K. Jacobson[b], and D. Papahadjopoulos[b], [a]Department of Physics, University of Maryland Baltimore County, Baltimore, MD, 21228, and [b]Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, NY, 14263

The recently developed fluorescence recovery after photobleaching (FRAB) techniques have been used to study the temperature dependence of the lateral mobility of fluorescent dyes, 3,3'-dioctadecyloxacarbocyanine, embedded in membranes prepared from egg phosphatidylcholine (EPC), dimyristoylphosphatidylcholine (DMPC), and dipalmitoylphosphatidylcholine (DPPC). A sharp change in recovery rate of fluorescent intensity after bleaching was observed between 21 and 24°C for DMPC and between 35 and 42°C for DPPC, which coincide with the liquid crystalline-gel phase transition temperatures of DMPC and DPPC respectively. From the recovery rate the diffusion constant D was deduced and found varying from $D \geq 5 \times 10^{-8}$ cm$^2$/sec at above the transition region to $D \leq 5 \times 10^{-10}$ cm$^2$/sec at below the transition region. For EPC no sharp change in fluorescent recovery rate was observed between 7 and 30°C, the measured diffusion constant varies from $8 \times 10^{-8}$ to $2 \times 10^{-7}$ cm$^2$/sec.

This investigation is supported by a grant CA-16743 (K.J.) awarded by the National Cancer Institute, DHEW.

W-POS-A17 THE STRUCTURE OF CHOLESTEROL-DIPALMITOYLLECITHIN MIXTURES AT LOW CHOLESTEROL CONTENT, PHASE SEPARATION AND BILAYER INTERACTIONS. L.J. Lis, N. Fuller, and R.P. Rand, Department of Biological Sciences, Brock University, St. Catharines, Ont. L2S 3A1

The interaction of low concentrations of cholesterol with dipalmitoyllecithin (DPL) in excess water was re-examined at various mole ratios using x-ray diffraction. At less than ca. 9 mole% cholesterol, two lamellar phases are observed below $T_m$ and one lamellar phase is observed above $T_m$. One structure observed below $T_m$, whose repeat distance stays close to 64 Å, is attributed to a solid DPL phase, while the other structure observed below $T_m$ increases in repeat distance with increasing cholesterol concentrations from ca. 64 Å to ca. 80 Å and is attributed to a solid DPL-cholesterol phase. At greater than 9 mole% cholesterol, only one lamellar phase is observed which decreases in repeat distance from ca. 80 Å to ca. 64 Å. This data disagrees with the present concept that low cholesterol contents (<11 mole%) simply cause a change in the DPL hydrocarbon chain tilt from tilted to perpendicular.(1) Rather, it is apparent that at these low cholesterol contents gross segregation of the lipid into regions of different structure occurs. At higher cholesterol contents, our findings agree with the existence of a single lamellar phase within which lateral phase separation in the plane of the DPL bilayer may occur.(2) We are exploring the cause of both the separation of the lipid into two structures and the large DPL lamellar repeat distances from the prospective of changing interbilayer interactions using a previously published method to measure forces between lipid bilayers.(3)

(1) D. Ladbrooke, R.M. Williams and D. Chapman (1968) Biochim. Biophys. Acta 150, 333-340.
(2) E.J. Shimshick, and H.M. McConnell (1973) Biochem. Biophys. Res. Comm. 53, 446-451.
(3) D.M. LeNeveu, R.P. Rand and V.A. Parsegian (1976) Nature 259, 601-603.

W-POS-A18 GLYCOPHORIN SPANS THE BILAYER. M.T. Tosteson and D.C. Tosteson, Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, Ill. 60637

Artificial membranes were exposed unilaterally to a glycophorin which had been extracted from human red cells. This produced an increase in the steady state membrane conductance proportional to the amount of glycoprotein added. Furthermore, the interaction of the protein with the bilayer promoted channel formation with a conductance of about $2.5 \times 10^{-10}$ mhos/channel. Addition of lectins to the cis-side produced rupture of the membrane, whilst hemoagglutinins added to the trans-side failed to produce an effect. Spectrin added to the trans-side lowered the membrane conductance induced by the protein. This effect was found to be reversible. It was further found that the sialoglycoprotein induces a change in surface potential. The maximum surface charge density (negative on the cis-side) computed from this potential was $1.7 \times 10^5$ charges/$\mu^2$. This surface charge was abolished by addition of neuraminidase to the cis-side. Addition of trypsin to the trans-side drastically changed the electrical properties induced by the sialoglycoprotein.

Evidence accumulated in the last years from studies performed on intact red cells and resealed ghosts tend to support the notion that glycophorin spans the membrane. There is also circumstantial evidence that it interacts with spectrin on the trans-side. The results of the present studies show that: a) glycophorin incorporates into lipid membranes with its N-terminal end (sialic acid and carbohydrates) on the cis-side and the C-terminal end on the trans-side. b) That the C-terminal end interacts with spectrin. c) That all the surface charge induced by the protein can be adscribed to the sialic acid residues.

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W-POS-A19 PROPERTIES OF BETA-HELICAL ION CHANNELS. S.J. Kennedy‡ H.R. Besch, Jr., A.M. Watanabe‡ A.R. Freeman and R.W. Roeske‡ Depts. Med. Biophys., Pharmacol., Biochem. & Med., Indiana University School of Medicine, Indianapolis, IN 46202.

Relaxation of the principle of equivalence, one of the original Pauling-Corey postulates, allows the generation of a new class of regular helical conformations of polypeptide chains. The two simplest members of this class, the $\beta^{\S}_{2,3}$- and $\beta^{\S}_{2,4}$-helices were previously described by Urry (PNAS 69:1610, 1972). To test the theory that new members of the class may be formed by polypeptide chains having appropriate amino acid sequences, a series of peptides having the sequence $(Leu\text{-}Ser\text{-}Leu\text{-}Gly)_n$ and expected to form $\beta^{12}_{6,6}$-helical ion channels in bilayer membranes was synthesized. Of these peptides, only the two theoretically capable of forming $\beta^{12}$-helices four turns in length altered conductance when tested in planar lipid bilayers. When added to the aqueous phases in concentrations of $10^{-6}$ to $10^{-5}$ M, both peptides increased the conductance by three to four orders of magnitude. The cationic transference number of the induced conductance was 0.82 in 1.0 M KCl. When the peptides were added in concentrations of $10^{-9}$ M, single channel current fluctuations were observed. Unit conductances were 1.45 nS, 0.67 nS and 0.43 nS in 1.0 M KCl, NaCl and LiCl, respectively. That polypeptide chains containing only L-amino acid residues can form β-helices is suggested by a unique $\beta^{13}_{4,9}$-helical model of the membrane penetrating segment of human erythrocyte glycophorin. A voltage-dependent shift in an equilibrium between $\beta^{13}_{4,9}$ and $\alpha_R$ conformations accounts for sodium channel activation kinetics and the associated gating currents which exhibit a rising phase. These characteristics suggest that the larger β-helices could be generally applicable to the structure of naturally occurring membrane ion channels. This work was supported in part by grants HL06308, HL05363, and HL07182 from NIH; by the Krannert Fund and by the Showalter Research Trust Fund.

**W-POS-A20 THE DIPOLE MOMENT OF A VOLTAGE DEPENDENT CONDUCTANCE PRODUCING ANTIBIOTIC-ALAMETHICIN. R.E. Yantorno[1] S. Takashima[1] P. Mueller[2], [1] Bioengineering Department, University of Pennsylvania, Philadelphia, Pennsylvania 19174 and[2] Department of Molecular Biology, Eastern Pennsylvania Psychiatric Institute, Philadelphia, Pennsylvania 19129.**

**The linear polypeptide antibiotic Alamethicin produces a voltage dependent conductance in an artifical bilayer membrane. The current produced for a voltage step is sigmoidal and is similar to that of the potassium system of the nerve. This investigation was conducted into one possible mechanism which could produce a voltage dependent conductance, the dipole moment.**

**The dipole moment of Alamethicin has been measured under various enviromental conditions e.g. from mediums which were hydrophilic to ones which were lipophilic. The data obtained supports and adds to the information available concerning the conformation of Alamethicin in these mediums ( Jung, Dubischar & Liebfritz, Eur. J. Biochem. 54:395,1975) as well as add information concerning the dipole moment of Alamethicin. A decrease in the effective length is observed as the lipophilicity of the medium is increased. This length change can be associated with an increase in the amount of alpha-helix content of this antibiotic. The value of the dipole moment is 67 Debye which is equal to two opposite charges separated by a distance of 14 A° . This value is much greater than that suggested as being required to produce the voltage dependent conductance which is observed ( Gordon & Haydon, Proc. Royal. Soc. Lond. 270B:433, 1975)**

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W-POS-A21 DISPLACEMENT CURRENTS IN ARTIFICIAL AND NATURAL PIGMENT-CONTAINING MEMBRANES. Felix T. Hong, The Rockefeller University, New York, N.Y. 10021

We have carried out experimental study of light-induced charge transfer across lipid bilayers which contain magnesium porphyrins and which separate a redox gradient of potassium ferricyanide and ferrocyanide (Hong & Mauzerall, Proc. Nat. Acad. Sci. 71, 1564 (1974)). Photoelectric responses in such systems can be separated into an interfacial AC component (displacement current) and a transmembrane DC component. The former is related to such fast photoevents as the early receptor potential (ERP) in visual membranes. The latter is important in the light-generated electric potential (proton gradient) across the membranes for use in forming ATP in chloroplast membranes and in purple membranes of Halobacterium halobium. Both the AC and the DC photoelectric responses are independent of the ionic conduction mechanism. The AC component is the manifestation of a novel chemical capacitance, which is related to pigment photoreaction and which can be shown to have a physical origin distinct from that of the ordinary membrane capacitance (Hong, Photochem. Photobiol. 24, 155 (1976)). The generality of the concept of chemical capacitance is demonstrated by the ability of our model to explain experimental data in the literature on a variety of pigment-containing membranes, both artificial and natural (Hong, J. Colloid Interf. Sci., Vol. 58, 1977, also to be reprinted in Colloid and Interface Science, Vol. 1, (Eds. M. Kerker, A.C. Zettlemoyer, & R.L. Rowell), Academic Press, New York, 1977, in press). Often, the displacement current is overshadowed by the simultaneously existing DC component but can be made conspicuous either by reducing the access impedance of the measuring device or by the action ionophores on the ionic membrane conductance. The concept of chemical capacitance is further generalized to offer an alternative mechanism of the ERP in terms of interfacial charge transfer. (Supported by NIH grant GM-20729)

**W-POS-B1** I-V RELATIONSHIPS OF TOAD BLADDER AND TOAD COLON. D.D. Macchia and S.I. Helman, Dept. of Physiology & Biophysics, Univ. of Illinois, Urbana, Ill. 61801.

Urinary bladders and colons of toads (Bufo marinus) were studied as sheets mounted between Lucite chambers and sealed with Sylgard 184. During a control period of up to 3 hours while the tissues were short-circuited continuously and bathed with a chloride-Ringer, the current-voltage relationships were determined at intervals of 20 minutes with a voltage clamp capable of changing the transepithelial voltage, $V_T$, for intervals of 600 msec in a range of -40 to +200 mV. In this range of voltage, the current responses were observed to reach stable values within 600 msec. When the data points were plotted, they were observed to fall into 2 regions of linear slope resistance, $R_1$ and $R_2$, intersecting at a voltage $E_1$ in the hyperpolarizing region of the I-V plots. The ratios of $R_1/R_2$ were 0.67 ± .05 (15) and 0.78 ± .06 (9) for bladders mounted stretched and unstretched, respectively, and for colons the ratio of $R_1/R_2$ was 0.63 ± .03 (8). The values of $E_1$ were 124.5 ± 4.7 (15) and 134 ± 3.0 (9) millivolts for stretched and unstretched bladders, respectively, and 109.5 ± 9.6 (8) mV for the colons. Studies of bladders were done with ADH (40 mU/ml) according to the methods described by Yonath and Civan to estimate the $E_{Na}$ of the sodium pump and the shunt resistance, $R_s$. In 12 studies, the values of $E_1$ and $E_{Na}$ were the same and their paired ratio was 1.01 ± .01 (12). Similarly, the values of $R_s$ were the same as the quotient $E_1/I_1$ where the current $I_1$ is the transepithelial current when $V_T = E_1$ ($(E_1/I_1)/R_s = 1.05 \pm .04$ (12)). Accordingly, it may be possible to estimate the $E_{Na}$ and $R_s$ of the toad bladder and perhaps the toad colon directly from studies of their I-V relationships as has been done previously in studies of the frog skin. (Supported by USPHS Grant AM 16663.)

**W-POS-B2** ABSORPTION OF NaCl BY RABBIT AND GUINEA PIG GALLBLADDER: EFFECT OF AMPHOTERICIN B. R.C. Rose, Departments of Physiology and Surgery, The Pennsylvania State University College of Medicine, Hershey, Pa. 17033

Active transport of ions at the basolateral cell membrane of gallbladder mucosa might be effected by either an energy-dependent, electrically neutral, coupled NaCl mechanism (J. Physiol. 161:474, 1962) or by a rheogenic Na pump. If the latter possibility is correct, the absence of a significant transmural potential difference (PD) during absorption could be due to coupling of Na and Cl transport at the mucosal membrane on a mechanism not dependent on cellular metabolism (J. Gen. Physiol. 65:769, 1975). Results of the present experiment in vitro might support one of the alternate views of the pump. K is required in the bathing solution (10 mEq/L) to have maximum rates of fluid absorption and $O_2$ consumption under control conditions, and to develop the maximum Amphotericin B-induced PD (7-20 mV, serosa positive). The effectiveness of alkali metal cations in maintaining the PD is K ≥ Rb > Cs > Li. In tissue samples with reduced K content, $O_2$ consumption was increased within 10 seconds after addition of K (+202%) or Rb (+225%) to the bathing media. Amphotericin B has the effect of uncoupling NaCl influx into the cell by increasing the mucosal membrane permeability to Na; influx of Cl is unaffected, and the coupled NaCl transport mechanism at the mucosal membrane appears to remain intact. The PD may result from a rheogenic Na (or Na-K) pump at the basolateral membrane. Since the tissue is unresponsive to Amphotericin B added to only the serosal bathing solution, it appears unlikely that the antibiotic alters the pump mechanism. Thus, a rheogenic pump may exist under control conditions also, but a transmural PD does not develop since influx of NaCl across the mucosal membrane is coupled. N.I.H. support.

**W-POS-B3** EFFECTS OF BUMETANIDE ON ACTIVE CL TRANSPORT AND OXYGEN CONSUMPTION IN THE ISOLATED BULLFROG CORNEA. H.F. Schoen* and O.A. Candia. Department of Ophthalmology, Mount Sinai School of Medicine of CUNY, New York, NY 10029.

Frog corneas were mounted in a modified Ussing chamber and short circuit current (SCC) and unidirectional Cl fluxes were measured. Bumetanide[1], a furosemide-type diuretic, at 0.01 mM, reduced the SCC from 0.48 ± .04 to 0.04 ± .01 $\mu$Eq/h.cm$^2$. The forward Cl flux declined from 0.71 ± .04 to 0.20 ± .03 (n=7), while, in separate experiments, the backward Cl flux did not change significantly (from 0.22 ± .03 to 0.23 ± .04; n=7). When corneas were mounted in a Cl-free Ringers and the net Na transport stimulated with amphotericin B, 0.01 mM bumetanide had no effect on the SCC. In separate experiments the effect of 0.01 mM bumetanide on $O_2$ consumption was measured in a Yellow Springs Instruments stirrer bath assembly. In a NaCl Ringers bumetanide decreased the $O_2$ consumption from 352 ± 14 to 297 ± 19 $\mu$l/h.cm$^2$ (15.5 ± 5.0%) (significantly different from sham-treated controls). This decrease is similar to that obtained with furosemide or when Cl is removed from the bathing medium. The results imply that bumetanide is a selective inhibitor of active Cl transport in the bullfrog cornea.

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[1]A gift of LEO Laboratories, Denmark.

**W-POS-B4** MECHANISMS OF ANION FLUX ACROSS ISOLATED RAT INTESTINAL BRUSH BORDER MEMBRANES. C.M. Liedtke* and U. Hopfer, Developmental Biology Center & Department of Anatomy, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

Anion transport into brush border vesicles was followed indirectly with a pH-electrode. Addition of sodium salts to a slightly buffered solution of membranes results in a rapid acidification of the medium due to $Na^+/H^+$ exchange between medium and intravesicular membrane space via the $Na^+/H^+$ antiporter (Murer et al. Biochem. J. 154, 597, 1976). Relaxation of the medium pH back to equilibrium is dependent on the anion flux into the membrane vesicles. Transport rates ($\tau^{-1}$ of the relaxation process in $min^{-1}$) were measured for $Cl^-$ ($\sim$ 10), $CH_3CO_2^-$ ($\sim$ 33), $SCN^-$ ($\sim$ 4), $NO_3^-$ ($\sim$ 5) and cyclamate$^-$ ($\sim$ .08). Of these anions only cyclamate$^-$ is relatively impermeant as indicated by a 120 fold smaller rate constant compared to $Cl^-$. Two modes of translocation can be distinguished for the permeant anions. Transport of $SCN^-$ and $NO_3^-$ are accelerated by an increase of proton conductance with carbonyl cyanide p-trifluoromethoxy phenylhydrazone (CF-CCP), thus, setting an upper limit for proton conductance in the absence of CF-CCP and suggesting membrane conductance for $SCN^-$ and $NO_3^-$. $Cl^-$ and $CH_3CO_2^-$ fluxes in the absence of CF-CCP are faster than can be explained by proton conductance, suggesting anion-proton co-transport. High $CH_3CO_2^-$ flux is a result of high membrane permeability to $CH_3CO_2H$. For $Cl^-$-$H^+$ co-transport to occur the existence of a symporter in the brush border membrane is postulated. A minimum of about 70% of the $Cl^-$ flux appears to be electroneutral co-transport with protons, while a minimum of about 50 and 70% of the $NO_3^-$ and $SCN^-$ flux, respectively, in the presence of CF-CCP is via an electrogenic route under our experimental conditions. $Cl^-$-$H^+$ co-transport in conjunction with a $Na^+/H^+$ antiport is consistent with an electrically silent NaCl absorption in the intact tissue.
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**W-POS-B5** TOAD URINARY BLADDER: ACTION OF METABOLIC INHIBITORS ON UNIDIRECTIONAL WATER FLUX, M.A.Hardy, Jr. and J.A.Funes.* Catedra de Biofisica, Facultad de Medicina, Buenos Aires, Argentina, (M.A.H. present address: Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33152.)

Metabolic inhibitors diminish the increase in water permeability elicited by antidiuretic hormone (ADH) and serosal hypertonicity in amphibian urinary bladder. In order to ascertain whether metabolic inhibitors act on basal water flux, mucosal-serosal tritiated water flux was measured in urinary bladders of the toad (Bufo arenarum). Administration of iodoacetate ($10^{-4}$ M), 2-4, dinitrophenol ($4\times10^{-4}$ M), sodium azide ($4\times10^{-3}$ M) or cyanide ($2\times10^{-3}$ M) on the serosal side increased the flux by 150-200% of the control. Maximal response was observed 5-10 min after introduction of the drug, returning to basal rates within 25-40 min. This increase was enhanced by theophylline ($10^{-2}$ M) when added together with the metabolic inhibitor or by ADH (10mU/ml), serosal hypertonicity (200 mM sucrose added to saline) and aspirin ($10^{-2}$ M) when added before the metabolic inhibitor.

One possible interpretation of these results would be that the action of metabolic inhibitors may be due to a rise in cell $[Ca^{2+}]$. An initially low $[Ca^{2+}]$ would increase mucosal permeability by itself and/or by stimulating adenyl-cyclase; further increase in $[Ca^{2+}]$ would reverse the effect, thus preventing stimulation by ADH and serosal hypertonicity.

**W-POS-B6** NICOTINAMIDE AND NICOTINIC ACID UPTAKE BY RAT RENAL CORTICAL SLICES. Karl R. Fox, Department of Pathology; University of Texas at Houston/Hermann Hospital; Houston, Texas 77025 and Department of Laboratory Medicine and Pathology; University of Minnesota; Minneapolis, Minnesota 55455 and David M. Brown, Departments of Laboratory Medicine and Pathology and Pediatrics; University of Minnesota; Minneapolis, Minnesota 55455.

In vitro slices of rat renal cortex accumulate [7-$^{14}$C] nicotinic acid by a saturable first-order mechanism. [7-$^{14}$C] nicotinamide is not similarly accumulated. Accumulation is significantly depressed by the incorporation of sodium azide 5mM or ouabain 1mM suggesting the process to be sodium-dependent. Accumulation of label is abolished by 2,4-dinitrophenal 0.1mM. We conclude that nicotinic acid, but not nicotinamide, is accumulated by in vitro rat renal cortical slice by a saturable energy-dependent first-order mechanism which has characteristics of specialized transport. Isonicotinic acid and pyridine-3-sulfonic acid, which have no capacity to substitute for nicotinic acid in vivo depressed transport slightly. 3-Methylpyridine, which can replace nicotinic acid in vivo, increased transport slightly. The effects of these added compounds may be related to interactions with cellular pyridine nucleotides. Depression by pyridine-3-sulfonic acid and/or isonicotinic acid may have been due to weak affinity for a common transport mechanism or antivitamin activity. Stimulation by 3-methylpyridine may have occurred by virtue of its vitamin activity.

W-POS-B7 PARALLEL PATHWAYS FOR NA AND Cl MOVEMENT ACROSS THE ISOLATED RAT GASTRIC MUCOSA: THE EFFECT OF ETHANOL. D.C. Dawson and A.R. Cooke*, Dept. of Physiology and Biophysics and Dept. of Internal Medicine, University of Iowa, and Veterans Hospital, Iowa City, IA 52242

Transmural unidirectional fluxes of $^{3}H$-mannitol and $^{22}Na$ or $^{36}Cl$ were measured simultaneously under short circuit conditions in portions of rat gastric mucosa stripped of serosal musculature. A plot of $J_{sm}^{Na}$ versus $J_{sm}^{man}$ can be described by a straight line with a zero intercept and a slope in accord with that predicted by the free diffusion coefficients of the isotopes. This result suggests that $J_{sm}^{Na}$ is largely confined to watery, non-cellular leak pathway marked by the transmural mannitol flux. Values of $J_{ms}^{Na}$, corrected for the Na flux through the leak path, are highly correlated with the short circuit current ($I_{sc}$). The relationships of $J_{sm}^{Cl}$ and $J_{ms}^{Cl}$ to the simultaneously measured mannitol fluxes suggest that a significant portion of both unidirectional Cl fluxes traverses a non-cellular leak path where Cl and mannitol move much as in free solution, but that there is a significant cellular component to both fluxes. If values of $J_{sm}^{Cl}$ and $J_{ms}^{Cl}$ are corrected for Cl flow via the leak path, the cellular component of both fluxes is correlated with $I_{sc}$. The tissue conductance can be described as having two components, a leak conductance linearly related to the transmural mannitol flux and an active conductance correlated with $I_{sc}$. The conductance of the leak path is in close agreement with that predicted from the movement of Na and Cl in the leak path. Ethanol (4%) on the mucosal surface increases the permeability of the leak path to Na, Cl and mannitol and inhibits the active transport of Na and Cl. The effect of ethanol is to increase the conductance of the leak path and decrease the conductance of the active path. NSF and NIH support.

W-POS-B8 THE DOGFISH GASTRIC MUCOSA: AN EXAMPLE OF ELECTROGENIC $H^+$ SECRETION George W. Kidder III, Dept. Physiology, Univ. Maryland Sch. Dentistry, Baltimore, MD and Mt. Desert Island Biol. Lab., Salsbury Cove, ME

Since the report of Hogben (Science 129:1224, 1959) that the chambered dogfish gastric mucosa secrets acid without generating a significant potential difference, this tissue has been regarded as a prime case of a coupled, neutral HCl pump. More recently, I have shown that this tissue, as mounted in 95% $O_2$/5% $CO_2$, is both hypoxic and hypocapnic, and that hyperbaric conditions equivalent to 190% $O_2$/10% $CO_2$ raise acid secretion ($J_H$) by 190% (to 5.5 $\mu Eq/cm^2 \cdot hr$), cause the PD to change from near zero to -5 mV (vs. mucosal =0), and cause a drop in transepithelial resistance from 425 to about 240 $ohm \cdot cm^2$. The mucosal-positive PD is unique to this tissue, being reversed from all other species reported. Since PD and $J_H$ are correlated ($r = 0.72$) in experiments at constant mucosal pH (pH-stat = 4.5), this PD would not seem to be an electrode tip-potential. Moreover, decreasing mucosal pH to $<2$ reverses the sign of the PD, opposite to an electrode artifact. Could this PD be a liquid-junction potential due to diffusion of HCl out of the secretory pits? This is at variance with the correlation of PD and $J_H$, as the pH of primary secretion should not be a function of $J_H > 0$, and it does not explain the resistance changes observed. Measurements of pH and PD in the dogfish in vivo give similar results. If this PD is genuine, it strongly supports an electrogenic $H^+$ secretory mechanism as the simplest explanation for the data. (Sup. by NSF PCM73-06699 to GWK and GB-28139 to MDIBL.)

W-POS-B9 DMO* IONIZATION AT THE CELL SURFACES OF THE FROG GASTRIC MUCOSA. Leopoldo Villegas, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, IVIC, Apartado 1827, Caracas, 101, Venezuela.

Knowledgement of ionization of the weak acid at the cell surface is important in nonionic diffusion studies. Effects of the pd and the pH on the DMO distribution and transmucosal fluxes were measured. In mucosae incubated in open circuit (pd = 32 ± 1 mv) at pH = 7.4, DMO reaches 0.94 ± 0.09 and 0.20 ± 0.03 of the tissue water from the serosal and the mucosal surface, respectively. Short circuiting pd or changing pH between 6.9 - 7.9 do not affect DMO volumes, indicating that no pd develops between the solutions and the extracellular compartments by effect of the externally applied current. Using DMO concentration of 1.5 μM, the unidirectional transmucosal fluxes measured (pH = 7.4) were 3.4 ± 0.3 (serosal to mucosal) and 30.1 ± 1.2 (mucosal to serosal) x $10^{-5}$ $\mu M/cm^2.h$. These fluxes were independent of transmucosal pd but significantly dependent on solutions' pH. Consequently, DMO must predominantly cross the mucosa in its unionized form. The change in the mucosal to serosal flux equals the change in the unionized DMO expected by lowering the pH of the extracellular mucosal compartment from 6.0 to 5.0. The pH of the mucosal extracellular compartment could be lower than that of the bathing solution due to the primary acid accumulation. Contrarily the serosal to mucosal flux change due to lowering the solutions' pH from 7.9 to 6.9 is far lower from the theoretical change in the unionized DMO. The $HCO_3^-$ efflux difficults the existence of a pH in the serosal extracellular compartment below that of the external solution. The pH effects on the fluxes, different at both surfaces, support the existence of a restricted serosal extracellular compartment, not reached by external buffer and with different diffusion properties than that of an aqueous solution.

W-POS-B10 REVERSAL OF SOMATOSTATIN INHIBITION OF GASTRIC ACID SECRETION BY DIBUTYRYL-CYCLIC-AMP. Prakash Kulkarni,* Florence Hoffman,* and Richard L. Shoemaker. Dept. of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, Alabama 35294

In vitro experiments were performed on Rana pipiens gastric mucosa using the pH stat technique. Addition of Somatostatin to the serosal bathing solutions of the gastric mucosa inhibited the acid secretion; the decrease in the acid rate was accompanied by an increase in transmucosal electrical potential and the tissue electrical resistance. Somatostatin (0.2µg/ml) inhibited the acid secretion stimulated by Pentagastrin ($2.8 \times 10^{-7}$M), whereas 8µg/ml of Somatostatin was required to inhibit the acid secretion stimulated by histamine ($10^{-6}$M). The acid secretion inhibited by Somatostatin (concentration up to 100µg/ml) was reversed by Dibutyryl-cyclicAMP (DB-cAMP) at $3.3 \times 10^{-3}$M. An increase in the concentration of Pentagastrin or histamine failed to restore the acid secretion rate to control levels in the presence of Somatostatin in the serosal solution. The gastric mucosa secreted acid upon the replacement of Somatostatin by either Pentagastrin or histamine in the serosal solution. These results indicate that DB-cAMP acts down the stimulatory pathway from Pentagastrin or histamine and that cAMP could be the final or common mediator of gastric secretion. NSF support.

W-POS-B11 RECTIFICATION AND NON-LINEAR OSMOSIS IN FROG SKIN. Y.T. Lau, and R.H. Parsons, Department of Biology, Rensselaer Polytechnic Institute, Troy, N.Y. 12181

Osmotic water influx (epi. to corium) and outflux across poisoned frog skin was measured for various hypertonic sucrose gradients (Ringer's solution containing 2mMKCN plus sucrose). Extracellular space (ECS) and cell volume were measured on the same skins.

| | Lp(µl/hr·cm²·0.1 Osm) | ECS(µl/mg dry wt) | cell volume(µl/mg dry wt) |
|---|---|---|---|
| 0E 100C* | 4.72±0.57(7) | 1.80±0.19 | 1.53±0.10 |
| 100E 0C | 3.92±0.27(6) | 1.47±0.10 | 1.71±0.09 |
| 0E 300C | 4.44±0.41(7) | 1.60±0.09 | 1.49±0.10 |
| 300E 0C | 3.21±0.22(6) | 1.09±0.07 | 1.14±0.11 |
| 0E 500C | 3.49±0.24(7) | 1.77±0.11 | 1.17±0.11 |
| 500E 0C | 2.40±0.16(7) | 1.07±0.08 | 0.98±0.10 |

*E=Epithelial bath, C=Corium bath; number indicate sucrose concentration added to Ringer's solution. Results indicate a larger ECS for influx and a smaller ECS for outflux which correlates well with the relatively high osmotic permeability (Lp) for influx and the relatively low Lp for outflux (rectification). Non-linear osmosis is also apparent for both directions of flow. The permeability decreases as gradient increases and correlates with the shrinkage of the cell volume. The data is consistent with the view that tissue permeability depends upon the bathing osmolarity and that both cell volume and ECS are involved in controlling the osmotic water permeability.

W-POS-B12 THE ELECTRICAL PROPERTIES AND ACTIVE CHLORIDE TRANSPORT OF THE OPERCULAR EPITHELIUM OF THE EURYHALINE TELEOST, FUNDULUS HETEROCLITUS. K.J.Degnan,* K.J.Karnaky,Jr.* and J.A. Zadunaisky, Depts.of Physiology and Ophthalmology, N.Y.U.Med.Center, New York N.Y.10016, and Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672.

The epithelium lining the gill chamber of *Fundulus heteroclitus* with a density of $10^5$ chloride cells/cm² was studied for the first time as a membrane, which offers great advantages over other gill preparations for membrane transport studies. The isolated opercular epithelium of 100% seawater-adapted *Fundulus* displayed a mean potential difference (p.d.) of 18.7± 1.2 mV (seawater side positive), a short-circuit current ($I_{sc}$) of 136.5±11.1 µA/cm², and a d.c. resistance (R) of 173.7±12.1Ω-cm² (mean ± s.e.m., N=60). $Cl^-$-free-solutions, anoxia ($N_2$), ouabain ($10^{-5}$M), furosemide ($10^{-3}$M) and thiocyanate ($10^{-2}$M) produced significant (P<0.01) decreases in the $I_{sc}$ and p.d., while amiloride ($10^{-5}$M) and amphotericin B ($10^{-5}$M) had very slight or no effects on these parameters. Theophylline ($10^{-4}$M) and $HCO_3^-$ (16 mM) had stimulatory effects on the $I_{sc}$ and p.d. The p.d. was a linear function of the $Cl^-$ with a Nernst slope of 28.3±2.1 and a more complex nonlinear function of the $Na^+$. In 100% $O_2$ the $^{36}Cl^-$ outflux (blood to seawater side) was 211.7±27.1 µA/cm² while the $^{36}Cl^-$ influx (seawater to blood side) was 48.9±10.0 µA/cm². This resulted in a net $^{36}Cl^-$ outflux of 162.8±30.9 µA/cm² which was very close to the mean $I_{sc}$ of 158.6±16.3 µA/cm² for these flux studies. The unidirectional $^{22}Na^+$ outflux and $^{22}Na^+$ influx were 32.2±3.3 µA/cm² and 34.8±4.1 µA/cm² respectively resulting in a small net influx of 2.6± 4.6 µA/cm². In 95% $O_2$:5% $CO_2$ the unidirectional $^{36}Cl^-$ and $^{22}Na^+$ fluxes were the same as those when gassed with 100% $O_2$. These chloride cells are identical to those of the gill and the isolated short-circuited operculum permits a biophysical approach to teleost osmoregulation.

**W-POS-B13** VISCOSITY: A TOOL FOR EXPLORING BULK FLOW PATHWAYS. D. Hare and A. Johnson*, Department of Medicine & Biophysics, Veterans Administration Hospital, State University of New York at Buffalo, Buffalo, N.Y. 14215

Many epithelial tissues are observed to have a volume flow($J_V$) which is a nonlinear asymmetric function of osmotic gradient($\Delta\pi$). Commonly this nonlinearity is attributed to an unstirred layer. In Necturus gallbladder, where $J_V$ was measured gravinometrically using the Bentley bag technique, the hydraulic conductivity($L_p$) for $J_V$ from mucosa to serosa ($J_V M \rightarrow S$) is found to decrease 50% when $\Delta\pi$, created by raffinose, is increased from 25 mOs/L to 180 mOs/L. The unstirred layer would have to be 2.0cm to account for such a drop with the $J_V$'s measured. With stirring of the serosal bath, $J_V M \rightarrow S$ is not changed. Rather than an unstirred layer it is proposed that increasing viscosity($\eta$) in the resistance pathway of osmotic flow may contribute to the nonlinearity. If $\eta$ is increased from 1.20cp to 1.89cp by the addition of raffinose to both baths composed of normal Ringers so that the final solution contains 390 mOs/L in the mucosa and 700 mOs/l in the serosa compartments, $J_V M \rightarrow S$ is proportionately decreased. This does not happen when the solution is made hypertonic with $Na_2SO_4$. With the addition of 14.0 mM PEG 6000 to both baths, $\eta$ is increased to 4.6cp and $J_V M \rightarrow S$ is proportionately decreased, but with the addition of 0.84 mM Dextran 83,000 increasing $\eta$ to 5.4cp there is no significant change in $J_V M \rightarrow S$, indicating $\eta$ changes alone do not necessarily change $J_V M \rightarrow S$. In these experiments the inulin and PEG spaces measured by tracer technique are comparable, while the dextran space is 40% smaller. One interpretation is that dextran 83,000 does not have access to any pathway offering significant resistance to bulk water flow while PEG 6000 does. Bulk flow studies using artificial membranes support this interpretation.

**W-POS-B14** THE ROLE OF MEMBRANE FLUIDITY ON THE PERMEABILITY CHANGES OF ADH STIMULATED TOAD URINARY BLADDER. B.R. Masters, D.D. Fanestil*, and J. Yguerabide, Depts. of Medicine and Biology, University of California, San Diego, La Jolla, California 92093

The microviscosity of single, isolated mucosal cells from toad urinary bladder of Bufo marinus was measured by the technique of polarized fluorescence emission using the probe perylene, which measures the viscosity of the hydrophobic region of the bilayer membrane. At 23°C., 5 mM dibutyryl c-AMP decreased the microviscosity from 3.31 P to 3.07 P (a decrease of 7.3%), and the anisotropy from 0.0719 to 0.0671. 8-Bromo c-AMP produced a similar decrease in the emission anisotropy. ADH at 200 mU/ml did not affect the microviscosity of the isolated cells, although it reduced the anisotropy of the perylene stained intact urinary bladder. An analysis of our results indicates that the detected decrease in the microviscosity is sufficiently large to account for the ADH changes in non-electrolyte permeability across the toad urinary bladder by a solubility-diffusion mechanism, but the changes are not large enough to account for the ADH induced change in water permeability.

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**W-POS-B15** THE APPLICATION OF THE KOEFOED-JOHNSON AND USSING MODEL TO K ACTIVE TRANSPORT IN INSECT MIDGUT. J. T. Blankemeyer* (Intr. N. Bashirelahi) Dept. of Physiology, Univ. of Maryland Sch. of Dentistry, Baltimore, MD 21201

The Koefoed-Johnson and Ussing (KJU) model (Acta Physiol. Scand. 42:298, 1958) was developed for the frog skin but has been extended to other systems. The model has had serious challenges with the most recent by Finn (Physiol. Rev. 56:453, 1976). The questions raised about the model suggested testing the model on the electrogenic K pump of insect midgut. The midgut, chamber mounted, actively transports K from the hemolymph-side to the lumen-side when K is the only alkali ion in the bathing solution. The potential difference (PD) is positive from lumen to hemolymph and is quickly diminished by anoxia as are the short-circuit current and the K influx. The PD changes from 145 mV at the start of an experiment to 25 mV after two hours. The decay of the PD has been shown to be independent of the passive conductance of the midgut and to be correlated with decay in the EMF of the K pump. If the slope of $\Delta PD/\Delta K$ changes (subst. choline)($[\Delta VzF/RT]\log K$; Nernst slope) is plotted vs. time the initial value is 59 mV, which decays to 20 mV after two hours. However, the Nernst slope in anoxia is only 2 mV for both sides whereas the 59 mV slope is for the hemolymph-side. The Nernst slope of 59 mV is found only at the start of an experiment when the EMF is maximal and is not found when the EMF of the K pump is reduced by time or anoxia. These results suggest that the Nernst slope is dependent on the pump EMF and independent of ion-electrode EMF postulated by the KJU model.

**W-POS-B16** AN ESTIMATE OF CORNEAL ENDOTHELIAL CELL ASHESION FORCE. J. J. Lim and J. Fischbarg, Dept. of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, N. Y. 10032

Corneal endothelial cell adhesion force has been estimated by measuring the critical hydrostatic pressure at which the rate of forward fluid flow cancels that of the backward fluid leak. Since the corneal endothelium consists of monolayer of cells, it is very suitable for theoretical and experimental analysis pertinent to the cell adhesion force. Under the assumption that the fluid leaks through the intercellular junction from the aqueous to the stromal side due to the hydrostatic pressure imposed to rupture the junction, the critical pressure measured would be related to the cell adhesion force. The pressure thus measured was about 150 cm of a water column, which corresponds to $1.47 \times 10^5$ dyne/cm$^2$. Since the concave surface, which is assumed to be parallel to the endothelial cell layer, of the cornea makes about $30^o$ with the horizontal plane, the adhesion force was obtained as $7.5 \times 10^4$ dyne/cm$^2$. This value compares very well with the one recently obtained for the force between lecithin bilayers. We have also measured the osmotic permeability and the rate of fluid transport across the corneal endothelium under various experimental conditions. The osmotic pressure estimated from the permeability and fluid transport measured agrees very well with the gravitational force of the water column. In addition, the effects of a few ions such as $Mg^{++}$ and/or $Ca^{++}$ ions on the critical pressure, thus the cell adhesion force, will be presented.

W-POS-C1 STOICHIOMETRY OF LECITHIN-CHOLESTEROL COMPLEXES. N. L. Gershfeld* (Intr. by V. A. Parsegian). Laboratory of Physical Biology, NIAMDD, NIH, Bethesda, Maryland 20014.

Temperature-composition phase diagrams for anhydrous mixtures of cholesterol (CHOL) with dimyristoyl (DML) and with dipalmitoyl (DPL) lecithin were obtained using differential scanning calorimetry (DSC). Complexes form with molar ratios for lecithin:CHOL of 2:1 and 1:2; they are stable up to 70°. When X(CHOL) < 0.33, two phases coexist: complex (2:1) plus pure lecithin; when 0.33 < X(CHOL) < 0.67 complexes (2:1) and (1:2) coexist as separate phases. The corresponding phase diagram in water for these mixtures was determined by DSC and isopycnic centrifugation in $D_2O$-$H_2O$ gradients. Aqueous dispersions were prepared by various methods (vortexing, dialysis, sonication) yielding identical results except as noted below. When X(CHOL) < 0.33 two lipid phases coexist: pure lecithin plus complex (2:1) where the properties of the lecithin phase are determined by whether the temperature is below or above $T_c$, the gel-liquid crystal transition temperature. Therefore complex (2:1) will coexist with gel state below $T_c$ and with liquid crystal above $T_c$. The densities follow in the order gel > complex (2:1) > liquid crystal. The density of complex (2:1) is less sensitive to temperature in the range 5°-45° compared to the temperature dependence for DML and DPL where large changes in density occur at $T_c$. When X(CHOL) > 0.33, CHOL phase coexists with complex (2:1); anhydrous complex (1:2) is apparently not stable in $H_2O$. The results are independent of the method and temperature used for preparing the lipid dispersions. However, when dispersions are prepared by sonication at $T > T_c$, an apparent 1:1 complex is formed. Evidence suggests the 1:1 complex is metastable.

W-POS-C2 THE CONTRIBUTION OF CALCIUM TO THE STRUCTURAL STABILITY OF MYELIN:AN X-RAY DIFFRACTION STUDY. Raúl Padrón*, T.Kirchhausen, L. Mateu*(IVIC,Apdo.1827,Caracas 101,VENEZUELA) and D.A.Kirschner*(Brandeis University,Waltham,Massachusetts 02154,USA).(Int.by L.Sananes).

Myelin displays the known property to swell in water.The kinetics of swelling and the study of the intermediate states may give information about the interactions which stabilize the spiral wrapping of the membrane around the axon.This information was obtained by small-angle X-ray diffraction using a position-sensitive proportional detector (10 min counting intervals).The native nerve displays the first 5 reflections of a 170 Å one-dimensional unit cell. The patterns of the same nerve after swelling shows the first 6 reflections of the 220 Å one-dimensional unit cell.Completion of the swelling takes several hours, and it is reversible:after several hours of reimmersion in toad ringer solution(with 1.9 mM $Ca^{++}$ or without $Ca^{++}$) the reassambled nerve displays a diffraction pattern which is nearly identical to that of native nerve.However,a new reimmersion in water reveals that the kinetics of swelling of nerves reassambled in ringer with calcium was very similar to that of native nerves,requiring several hours;whereas the kinetics of swelling of nerves reassambled in ringer without calcium is much faster,requiring some minutes.Our results are consistent with the interpretation that calcium participates in the interaction between adjacent membrane pairs at the level of intraperiod line.

W-POS-C3 MONTE CARLO STUDIES OF PHOSPHOLIPID LAMELLAE. H. L. Scott, Department of Physics, Oklahoma State University, Stillwater, Oklahoma 74074

We present the results of a series of Monte Carlo computer experiments which simulated systems of hydrocarbon chains with one end attached to a plane interface and the remainder of the chains constrained to lie below this plane. In the experiments we made use of the rotational isomeric model to generate configurations for a finite number of chains placed in a fixed volume with periodic boundary conditions. The chains interacted with each other via hard core repulsive forces only. Using standard Monte Carlo importance sampling techniques we then determined average order parameters for each chain position. In the computer runs in which the lipids were packed relatively closely, the plots of order parameter vs. carbon number resembled the results of NMR experiments.[1] When the area per molecule was larger, the plots resembled more closely the results of spin-label experiments.[2] This suggests that discrepancies in the two sets of experiments is due to the spin labels' presence, effectively increasing, at least locally, the area per molecule. Also, our results show that, for the determination of the thermodynamic states of the hydrocarbon region of a monolayer or bilayer, the hard core repulsive forces are the most important. We also present preliminary results of a Monte Carlo study of the head group-water interface.

1. J. Seelig and W. Niederberger, 1974, Biochemistry 13, 1585-1588.
2. W. L. Hubbel and H. M. McConnell, 1971, J. Am. Chem. Soc. 93, 314-326.

**W-POS-C4** TEMPERATURE-DEPENDENT BEHAVIOR OF NEAT CHOLESTERYL LINOLEATE AND A CHOLESTERYL LINOLEATE-TRIOLEIN MIXTURE: A $^{13}C$ NMR STUDY. J.A. Hamilton*, N. Oppenheimer*, and E. H. Cordes*. Intr. by J. Morrisett, Dept. of Chemistry, Indiana Univ, Bloomington, IN 47401.

Natural-abundance Fourier transform $^{13}C$ NMR spectra at 25.2 MHz were obtained for neat cholesteryl linoleate (CL) between 33° and 46°C. Spectra obtained in the isotropic phase (>35°) are characterized by numerous narrow single-carbon resonances arising from both the linoleic acid moiety and the cholesterol ring and aliphatic side chain. In the cholesteric mesophase (34°) resonances from carbons of the cholesterol ring and its aliphatic side chain, and of the fatty acid carbonyl and C-2 are broadened beyond detectability. The fatty acid olefinic resonance and resonances from carbon atoms close the terminal end of the fatty acid chain remain narrow. In the smectic mesophase (33°) the above resonances observed at 34° are broadened considerably. As the isotropic → cholesteric phase transition for CL is approached from 46°, resonances attributable to cholesterol ring carbons broaden differentially, while the fatty acid resonances do not broaden appreciably. In particular, the cholesterol C-3 methine resonance exhibits a more rapid broadening than the cholesterol C-6 methine resonance. Since these carbon atoms are part of a fused ring system and their C-H vectors are in orthogonal directions, this result demonstrates that rotations of the cholesterol ring system become increasingly anisotropic in the isotropic phase as the cholesteric phase transition temperature is approached. With a ternary mixture of ∿58% CL, 20% cholesteryl oleate and 22% triolein (w/w/w), no phase transitions were observed visually between 50° and 30°, before crystallization of the sample occurred. $^{13}C$ NMR spectra of this isotropic melt revealed narrow resonances for carbon atoms of the fatty acid moiety, and cholesterol ring and its aliphatic side chain at all temperatures and a much smaller increase in the C-3/C-6 linewidth ratio as a function of decreasing temperature.

**W-POS-C5** FLUORINE-19 NMR STUDIES OF PHASE TRANSITION OF ESCHERICHIA COLI MEMBRANES. M.P.N. Gent* and C. Ho, Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pa. 15260

$^{19}F$ nuclear magnetic resonance (NMR) spectroscopy has been used to measure the molecular motions of the phospholipid fatty acids in bacterial membranes. Myristic acids labeled at specific positions with a difluoromethylene group are biosynthetically incorporated into the membranes of wild type, K12(λ), and an unsaturated fatty acid auxotroph, L4-11, of E. coli. Variation of the growth temperature and the fatty acid supplement allows manipulation of the lipid composition so that various membrane phase transition temperatures can be reached. The line shape of the $^{19}F$ resonance is used to measure of the order parameter of the difluoromethylene group of the phospholipids. The phase transition in bacterial membranes and synthetic lipid membranes, consisting of pure lipid multilayer suspensions containing a small amount of difluoromyristate, have been studied by this technique. The resonance becomes very broad and unobservable below the phase transition. Above the transition only a slight narrowing with temperature occurs in synthetic lipid membranes. In bacterial membranes significant narrowing also occurs at about 50°C. The results of this method are compared to results of methods, such as electron spin resonance of nitroxide probes and fluorescence studies. (This work is supported by research grants from NSF and NIH.)

**W-POS-C6** AN INTENSIFIER-VIDICON SYSTEM FOR RECORDING X-RAY DIFFRACTION PATTERNS FROM WEAKLY DIFFRACTING BIOLOGICAL STRUCTURES.† S. G. Gruner*, J. R. Milch, R. D. Piccard*, Geo T. Reynolds. Department of Physics, Princeton University, Princeton, N. J. 08540

Many important X-ray diffraction studies of biologically active structures do not permit the long recording times required by conventional film or diffractometer detectors. Included in this category are, for example: studies of membranes exhibiting a high degree of motional freedom; investigation of dynamic structural changes in muscle; and single crystal studies of proteins which are difficult to prepare, and which are structurally changed during the exposure to the X-ray beam. By combining image intensification and computer controlled vidicon recording, a system has been developed which produces diffraction data limited only by the statistics of the incident X-ray flux. Each diffracted X-ray contributes to the integrated two dimensional pattern stored digitally on disk for computer processing. By suitably controlling image intensifier gain and integration times, the system can be optimized to measure the intensities in the diffraction pattern to the required accuracy in the shortest possible time. This varies with the detailed nature of the pattern, and reduction in time by factors of 30 to several hundred have been achieved. Computer corrections are made where necessary for non-uniformities and spatial distortion introduced by the recording system. An important feature of the technique is that it is not count rate limited, and therefore is particularly suited for use with high intensity beams from synchrotron sources. The system has been applied successfully to several biological systems, and results will be presented from studies of t-RNA aminoacyl ligase, and frogs eye rod outer segment disk vesicle membrane. †Supported by ERDA, DBER, Contract AT (11-1) - 3120

W-POS-C7 RAMAN SPECTRA OF CHOLESTERYL ACETATE - PHOSPHATIDYLCHOLINE COMPLEXES. S. C. Goheen, L. J. Lis, and J. W. Kauffman, Bioengineering Center, Northwestern University, Evanston, Ill. 60201. Dept. Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1, Canada.

We have used Raman spectroscopy to examine the effect of cholesteryl acetate on the liquid crystallinity of the dimyristoyl phosphatidylcholine - water system. Laser Raman spectra in both the C-C and C-H stretch regions have been obtained for several mixtures of dimyristoyl phosphatidylcholine and cholesteryl acetate at room temperature. These spectra show that cholesteryl acetate disrupts the packing order of the hydrocarbon chains at very low concentrations. At some critical concentration (ca. 12 mole percent) the hydrocarbon chains become more ordered and the cholesteryl acetate appears to form crystalline regions that remain in suspension with the lecithin. We suggest that these results will provide information which can be related to other model systems which have been compared to atherosclerotic plaque.

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W-POS-C8 LASER-RAMAN ANALYSES OF THERMOTROPIC CHANGES IN BIOMEMBRANES AND MODEL PROTEIN-LIPID MEMBRANE SYSTEMS. Surendra P. Verma* and Donald F. H. Wallach, Tufts-New England Medical Center, 171 Harrison Ave., Boston, Mass. 02111.

We have used Raman spectroscopy in the CH-stretching region ($2700cm^{-1}$ - $3000cm^{-1}$) to detect thermotropic transitions in erythrocyte ghosts and model membrane systems. The relative intensities (I) of the strong CH-stretching bands at $2850cm^{-1}$, $\sim 2890cm^{-1}$ and $\sim 2930cm^{-1}$ are temperature dependent. For erythrocyte ghosts, plots of the relative-intensity ratios, i. e. $[I_{2890cm^{-1}}/I_{2850cm^{-1}}]$ and $[I_{2930cm^{-1}}/I_{2850cm^{-1}}]$ vs. temperature reveal discontinuities at approximately -4°C and 17°C, and at -8°C and 42°C, respectively (at pH 7.0-7.4). The high-temperature transition of $[I_{2930cm^{-1}}/I_{2850cm^{-1}}]$ is very pH-sensitive, drops to 24°C at pH 6.5. and at pH 6.0 merges with the -8°C step. The thermotropism below 20°C is assigned to membrane lipids that at high temperature is attributed to membrane proteins (i) by virtue of the fact that the change is due primarily to changes in $CH_3$-stretching of amino acid residues and (ii) because we obtain similar changes of $CH_3$-stretching during reversible unfolding of soluble proteins in acid aqueous media. To clarify the multiple thermotropic transitions in biomembranes, we have studied the effect of inserting melittin and proteolipid apoprotein, proteins that interact through apolar interactions with paraffin chains, into dimyristoyl lecithin (DML) bilayers. We find that melittin, at a protein/DML ratio of 1/56 shifts the lipid transition temperature from 23°C to 34°C. In contrast, proteolipid apoprotein produces a complex effect: Some DML (presumably bulk phase) goes through a sharp transition at 23°C. The rest shifts state gradually between 25°C and 40°C and presumably represents boundary layer lipid.

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W-POS-C9 LIPID STRUCTURE OF THE CELL MEMBRANE OF *THERMOPLASMA ACIDOPHILA*. Li L. Yang and A. Haug*, Department of Biophysics, Michigan State University, and MSU/ERDA Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

The thermophilic acidophilic mycoplasma *Thermoplasma acidophila* grows optimally at pH 2 and 56°C. Since the membrane comes into direct contact with this harsh environment, the structural and functional characteristics of the membrane may determine the environmental limits at which *T. acidophila* grows. Therefore, we analyzed the membrane lipid components which may contribute to the rigidity of this membrane as determined by electron paramagnetic resonance spectroscopy. The lipids of this protein-rich (75% w/w) membrane were studied by thin layer chromatography. The ratio of neutral lipid:glycolipid:phospholipid was 1:1:3 (w/w), where one phospholipid TLC band accounted for about 50% of the total lipids. Gas chromatographic and mass spectroscopic investigations of the lipids showed the presence of large quantities of long chain alkyl glyceryl ether, but very few fatty acid esters (0.3% w/w). Glycolipids comprised predominantly (70% w/w) monoglycosyl ether-linked diglycerides. Highly charged head groups were found in 80% of the phospholipids. In order to further understand structural and functional properties of the membrane, *T. acidophila* was adapted to growth at 37°C. For such membranes, the ratio of ether to ester linkages was decreased, while the ether chain length was shortened.

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W-POS-C10 CORRELATION BETWEEN THE EFFECTS OF VARIOUS STEROLS ON THE ARRANGEMENT OF SPIN PROBES IN MULTIBILYERS AND ON PERMEABILITY. K.W. Butler, I.C.P. Smith and M. Taylor*, Biological Sciences Division, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6

Liposomes have been extensively employed in studies of lipid bilayer permeability and in NMR and EPR studies of lipid structure. However, no correlation was found between the effects of including various sterols in liposomes on permeability to sodium and their effects on the spectra of intercalated fatty acid EPR probes. Because it is difficult to differentiate between effects on molecular order and effects on molecular motion using EPR spectra of probes in liposomes, we turned to studies of oriented planar multibilayers. It was found that both permeability decreasing and permeability increasing sterols caused increases in the rotational correlation time of the spin probes. Sterols that decreased permeability increased the tendency of the probes to align themselves close to the normal to the bilayer, sterols that increased permeability made the orientation of the probes more random,and sterols which had little effect on permeability had little effect on probe orientation. It is concluded that this orientation effect, which is obscured in liposome studies by the correlation time effects, is related to permeability regulation by sterols.

W-POS-C11 THEORETICAL ANALYSIS OF THE VISIBLE ABSORPTION AND CIRCULAR DICHROIC SPECTRA OF THE PURPLE MEMBRANE FROM HALOBACTERIUM HALOBIUM. T.L. Hsiao, D.D. Muccio and J.Y. Cassim, Department of Biophysics, The Ohio State University, Columbus, Ohio 43210.

The absorption and circular dichroic spectra of the purple membrane from Halobacterium halobium consists of a pair of oppositely signed circular dichroic bands centered near an absorption maximum. Calculations employing the molecular exciton model and based on the arrangement of bacteriorhodopsin molecules in clusters of three with a three-fold axis of symmetry (cyclic trimer) have been made in order to determine if such a model is consistent with experimental results. Computations were carried out with an optimization curve fitting program written in Fortran IV. Calculations indicated that in the case of cyclic trimer, exciton interaction would result in two exciton bands with one being doubly degenerate. The computation procedures were as follows: (1) the experimentally determined transition dipole strength of the bacteriorhodopsin chromophore was used to generate the circular dichroic spectrum based on the cyclic trimer exciton model, (2) two adjustable parameters, the positions of the transition dipoles and distance between transition dipoles were adjusted until a "best fit" occurred between the calculated and experimental circular dichroic spectra and (3) using these "best fit" values of the adjustable parameters the absorption spectrum was generated and shown to be in good agreement with the one determined experimentally. The "best fit" value of the distance between the retinals within the protein clusters was about 7Å which is in good agreement with present estimates based on experiment. However, if the "best fit" parameters were determined from the absorption spectrum, the circular dichroic spectrum generated did not fit the experimental one as well as the previous procedure.

W-POS-C12 SPATIAL LOCALIZATION AND ORIENTATION OF THE CHROMOPHORE OF BACTERIORHODOPSIN. G. I. King, R. A. Bogomolni, S.-B. Hwang and W. Stoeckenius, Cardiovascular Res. Inst. and Dept. Biochemistry & Biophysics, University of California, San Francisco, CA 94143; Ames Res. Ctr., NASA, Moffett Field, CA 94035. B. P. Schoenborn, Dept. Biology, Brookhaven Nat. Lab., Upton, New York 11973.

Deuterated retinal, extracted from the purple membrane of *H. halobium* grown in totally deuterated medium (a gift from H. Crespi, Argonne National Laboratory), was used to reconstitute the "chromophore free" apomembrane of normal bacteria. Analysis of neutron diffraction data obtained from this substituted material and from that of membrane reconstituted with normal retinal permits the determination of the retinal position. The difference between the neutron density profiles of deuterated and normal sample corresponds to the position of the beta ionone ring of retinal, where the largest density of hydrogen atoms occurs. The distance between ionone rings in the plane of the membrane is about 18.6 A. The position of the ring across the membrane is found at about 1/3 the membrane thickness from one surface. Complete localization of the chromophore requires knowledge of the angle of tilt with respect to the membrane plane and the angular orientation in the plane. The tilt of the transition dipole moment has been determined to be 23.5 ± 2° from linear dichroism measurements on multilayers of purple membrane (see abstract above). The angular orientation in the membrane plane is estimated from circular dichroism data on chromophore exciton interaction. A model consistent with this data and with the reported sequences of retinal containing polypeptides will be presented.

W-POS-C13 ORIENTATION OF THE BACTERIORHODOPSIN TRANSITION DIPOLE. R. A. Bogomolni, S.-B. Hwang, Y.-W. Tseng*, G. I. King and W. Stoeckenius, Cardiovascular Research Institute and Department of Biochemistry & Biophysics, University of California, San Francisco, CA 94143 and Ames Research Center, NASA, Moffett Field, CA 94035.

Purple membrane of *H. halobium* mixed with phospholipids was oriented in molecular films in an air-water interface. Around 30% of the film surface is occupied by the purple membranes and over 90% of them orient with the membrane outer surface towards the air. Multilayers of this material, when examined by low-angle X-ray diffraction, show a maximum deviation from planarity of about 2°. The angular orientation of the bacteriorhodopsin transition dipole was obtained from these uniaxially symmetric samples by three different techniques with constant results, namely measurement of: (a) linear dichroism, (b) optical density of the oriented sample vs. its isotropic suspension, (c) absorbance of the oriented sample vs. the calculated absorbance of an isotropic sample of identical molecular concentration. This concentration is determined from the fractional area covered by purple membrane (as measured by electron microscopy) and the crystallographic unit cell dimensions. The light-adapted chromophore's transition dipole (568 nm) is tilted 23.5 ± 2° with respect to the membrane plane. The measured angle for dark-adapted purple membrane ($\lambda_{max}$ 560) is about 22°, a very similar value in spite of the differences in isomeric states. Considerations based on the similar tilt angles and the circular dichroism spectra of the two forms suggest that the chromophore is spatially constrained by a rigid protein binding site.

W-POS-C14 ANGULAR ORIENTATION OF AN INTERMEDIATE OF THE BACTERIORHODOPSIN PHOTOCHEMICAL REACTION CYCLE. S.B. Hwang, R.A. Bogomolni, Y.W. Tseng and W. Stoeckenius. Cardiovascular Research Institute, Department of Biochemistry and Biophysics and Department of Physiology, University of California, San Francisco, CA 94143, and NASA Ames Research Center, Moffett Field, CA 94035.

Bacteriorhodopsin oriented in purple membrane multilayers(dried under normal atmospheric water vapor pressure) shows the typical absorption spectrum in its light adapted form with $\lambda_{max}$=568 nm, and undregoes a normal dark adaptation with a dark adapted $\lambda_{max}$=559 nm. However the photochemical cycle of purple membrane in the multilayers is significantly slower than in aqueous suspensions. The time for half-decay of the light-induced absorbance measured at 420 nm or at 540 nm is about 1 sec.(1 msec. actinic flash at 575 nm), and no transient overshoot at 660 nm is detected. This would be indicative of intermediate O (640). Continuous illumination of the sample with light of wavelength longer than 600 nm allowed us to simultaneously scan spectra from 580 nm to 360 nm. A set of spectra at varying actinic light intensities shows an isosbestic point at 456 nm, suggesting only two species in photochemical equilibrium, namely: bR(568) and M(409). Addition of the photoconverted fraction of the bR spectrum (calculated from the absorbance change at 568 nm) to the difference spectrum yields the spectrum of the intermediate M in the multilayers. This spectrum shows no detectable absorption between 580 nm and 500 nm and peak with $\lambda_{max}$=409 nm. Spectra of M measured with polarized light permited the calculation of the linear dichroism, and thus the angle of tilt of the transition dipole, for the 409 nm absorption band. The calculated angle does not differ significantly from that obtained for the bR (568) chromophore.

W-POS-C15 CD AND TRANSIENT DICHROISM STUDIES OF BACTERIORHODOPSIN: MEASUREMENTS OF PROTEIN ROTATION AND CHROMOPHORE ORIENTATION. M. P. Heyn, Dept. of Biophysical Chemistry, Biozentrum, CH-4056 Basel, Switzerland, and R. J. Cherry*, Lab. für Biochemie, ETH, CH-8092 Zürich, Switzerland.

The bacteriorhodopsin (BR) molecules are arranged within the purple membrane (PM) in a rigid hexagonal array. Exciton coupling effects between neighboring chromophores are observed in the CD spectra and the anisotropy of the flash-induced linear dichroism (LD) decays with a time constant characteristic for the slow rotation of whole PM patches. Upon the addition of 5 °/o ether the CD exciton bands disappear and a fast component appears in the decay of the anisotropy. Since electronmicroscopy and light scattering experiments show that no fragmentation of the membrane occurs upon the addition of ether, the simplest explanation of these observations is that ether disassembles the protein lattice, allowing rotational motion of the BR molecules. Similar observations were made when BR was incorporated into lipid vesicles. Since the rapid rotational component does not lead to a complete decay of the anisotropy, the motion occurs around an axis normal to the plane of the membrane, which explains the disappearance of the exciton CD bands. From a measurement of the residual anisotropy the angle between the transition dipole moment and the plane of the membrane can be determined. The same information can be obtained from an analysis of the LD of oriented PMs. Additional information on the transition dipole moment geometry is contained in the exciton CD amplitude. On the basis of these measurements a model will be presented for the position and orientation of the chromophores in the PM.

**W-POS-C16** COMPARATIVE STUDIES OF THE BLEACHING AND REGENERATION OF PURPLE MEMBRANE FROM HALOBACTERIUM CUTIRUBRUM AND HALOBACTERIUM HALOBIUM. G.K. Papadopoulos, T.L. Hsiao and J.Y. Cassim, Department of Biophysics, The Ohio State University, Columbus, Ohio 43210.

Purple membrane preparations from Halobacterium cutirubrum and Halobacterium halobium have been bleached sequentially by exposures to light in the presence of hydroxylamine, with concomitant monitoring of their absorption and circular dichroic spectra. Such preparations were then sequentially regenerated by incubation with all-trans retinal. A comparison of the absorption and circular dichroic spectra of the bleaching and regenerating purple membrane from both species indicates that the underlying mechanisms for these processes are essentially the same, if not identical. Furthermore, perturbation of the purple membrane of either species with Triton X-100 leads to the abolition of the two visible circular dichroic bands of oppositely signed ellipticities and the appearance of a single positive band with a peak at about the absorption maximum. Such results taken in conjunction with the results of comparative spectral studies of both membranes done in this laboratory*, can be interpreted as indicative of a similarity, if not essential identity, in the fine structure of the purple membrane of both species. Since bleaching and regeneration studies of the purple membrane of Halobacterium halobium are consistent with an exciton interaction of the retinal chromophores in the membrane, the same mode of interaction can be suggested for the membrane of Halobacterium cutirubrum.

*Muccio, D.D., T.L. Hsiao and J.Y. Cassim, "Comparative spectral studies of the purple membrane from Halobacterium cutirubrum and Halobacterium halobium", these abstracts.

**W-POS-C17** COMPARATIVE SPECTRAL STUDIES OF PURPLE MEMBRANE FROM HALOBACTERIUM CUTIRUBRUM AND HALOBACTERIUM HALOBIUM. D.D. Muccio, T.L. Hsiao and J.Y. Cassim, Department of Biophysics, The Ohio State University, Columbus, Ohio 43210.

Direct comparison of the absorption and circular dichroic spectra (200-700 nm) of dark- and light-adapted purple membrane from Halobacterium cutirubrum and Halobacterium halobium indicates no apparent species differences. Based on our recently published analysis of the spectra of the purple membrane from Halobacterium halobium,* it is concluded that: (1) no major differences exist in the molecular organization and protein structure of the two purple membranes, (2) if exciton interaction between the retinal chromophores is a reasonable possibility in the case of the purple membrane from Halobacterium halobium, it must be similarly so in the case of the membrane from Halobacterium cutirubrum and (3) the effects of light adaptation on the membrane structure of both species are essentially the same. (Supported in part by an Ohio State University Small Research Grant.)

*Becher, B., and J.Y. Cassim. 1976. Effects of light adaptation on the purple membrane structure of Halobacterium halobium, Biophys. J. 16:1183.

**W-POS-C18** PHOTODYNAMIC EFFECTS ON LIPID REGIONS OF YEAST CELLS. G.E. Cohn, J.M. Collins* and B.S. Devitt*, Biophysics Laboratory, Physics Department, Illinois Institute of Technology, Chicago, IL 60616.

The yeast Saccharomyces cerevisiae can be inactivated in aqueous suspension by visible light ($\lambda > 400$ nm) in the presence of the non-penetrating dye eosin Y. The kinetics of inactivation indicate that singlet oxygen generated by extracellular eosin is the principal damaging species. Spin label ESR spectroscopy of the hydrocarbon probes 7N14 and $12NS_{me}$ added to yeast has been employed to identify any related changes in their lipid-containing regions. Survival depends on the growth stage of the culture, with inactivation almost completely suppressed in cultures which have grown beyond exponential phase. The hyperfine splitting for $12NS_{me}$ in unirradiated yeast decreases as the cells pass through exponential phase, indicating that this probe changes its location from a polar to a hydrocarbon environment. However, 7N14 localizes in a nonpolar environment for all growth phases. Increasing the sample temperature during the irradiation of exponential phase cells from 19°C to 30°C increases the inactivation rate by a factor of 9, which was paralleled by a 50% decrease in tumbling time ($\tau_0$) for both labels. $\tau_0$ for each of these probes increased following photodynamic inactivation. These results indicate that the lipid regions of yeast, particularly the plasma membrane, are a target of eosin-sensitized photodynamic inactivation.
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W-POS-C19 CHARACTERIZATION OF RHODOPSIN DERIVED FROM PAPAIN PROTEOLYZED BOVINE ROD OUTER SEGMENT DISCS. Arlene D. Albert* and Burton J. Litman, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22901.

Rhodopsin, in sonicated resealed bovine retinal rod outer segment discs, was proteolyzed by treatment with papain for 60 minutes at 37°. This treatment removes about 30% of the mass of the protein, leaving a core fragment of approximately 26,000 daltons. Electron microscopy indicates that both the proteolyzed and control disc membrane suspensions were in the form of closed vesicles. The fluorescence anisotropy of the probe 1,6-diphenyl-1,3, 5-hexatriene (DPH) was determined over a temperature range of 5° to 48° in control and papain treated disc membrane suspensions. These results indicate that a reduced fluidity in the hydrocarbon region of the membrane is produced by papain treatment. The circular dichroism (CD) of intact and cleaved rhodopsin, fully solubilized in the detergent octyl glucoside, was measured in both the far ultraviolet and the visible spectral regions. Proteolysis produced little change in the visible CD spectra, indicating that the retinal binding site remains essentially intact. The far ultraviolet CD spectra demonstrates that the core fragment (the portion presumed to be embedded in the hydrocarbon region of the membrane) retains more than 90% of the helical content of intact rhodopsin. Thus while proteolysis appears to have an overall effect in reducing the membrane fluidity, the spectral properties of rhodopsin remain essentially unchanged by this treatment.
(Supported by U.S.P.H.S. Grant EY00548 and NSF Grant GB-41313.)

W-POS-D1 A NANOSECOND PHASE-SHIFT FLUOROMETER USING LASER ILLUMINATION AND A TRANSVERSE MODE ELECTROOPTIC MODULATOR. I. Salmeen and L. Rimai, Engineering & Research Staff, Ford Motor Co., Dearborn, MI 48121

We describe a simple phase-shift fluorometer using continuous laser excitation. The laser enables the use of a transverse mode electrooptic modulator with a half-wave retardation voltage of about 200 volts (in contrast to many kilovolts of longitudinal modulators) at frequencies up to 100 MHz. The modulated fluorescence signal is detected, after passing through a double monochromator, by a photomultiplier tube feeding an RF tuned amplifier. The RF phase is then determined by phase sensitive detection using a double balanced mixer with the reference obtained from a PIN photodiode-tuned amplifier combination which detects light split off from the main exciting beam. The laser and double monochromator enable the observation of modulated Raman scattering which is a convenient signal for determining the zero reference phase. Using 30 MHz modulation, we measure 0.43 $\pm$ 0.2 nsec for the lifetime of fluorescein in 0.5 M KI. Spencer and Weber (Ann. N.Y. Acad. Sci, 158, 361 (1969)) measure 0.49 nsec for a similar sample. This instrument may also be used to measure Raman spectra in the presence of fluorescence provided the fluorescence lifetime is longer than ca. 3 nsec.

W-POS-D2 LASER CONTROLLER FOR INTERFEPOMETRY IN THE ULTRACENTRIFUGE. Thomas M. Laue*, Richard Domanik, David Rhodes* and David A. Yphantis, Biochemistry and Biophysics Section, University of Connecticut, Storrs, CT 06268

A system to control the firing of a pulsed argon ion laser for interferometry in the analytical ultracentrifuge has been designed and assembled. Light through a counterweight or rotor reference hole provides synchronization pulses at the rotor frequency $\omega$. A novel, wide range, high precision phase-locked loop circuit generates pulses at a frequency of 4096 $\omega$. Appropriate circuitry then counts these pulses and fires the laser at the preselected fraction of a revolution and within the duty cycle limitations of the laser. The system also includes a Fabritek MP-12 minicomputer for the bookkeeping associated with multiplexing of the interference fringes from the reference and the selected cell, with control of photographic exposures, with selection of laser wavelength, etc. The controller locks onto the rotor rotation over the full speed range of the ultracentrifuge (from 800 to 68,000 RPM) and provides a resolution of 0.1° rotation of the rotor. Changes in speed are tracked and the device maintains synchronization during maximum acceleration of our lightest rotor (An-D). Currently the reference timing is provided by the standard light absorption scanner. Accordingly changes in the axis of rotation with speed induce systematic timing variations. Timing pulses derived solely from the interferometric optical system should prove immune to such shifts.
(Supported by NIH grant #AM-18001.)

W-POS-D3 AUTOMATED TELEVISION BASED IMAGE MEASUREMENT SYSTEMS. R. A. Domanik, Department of Chemistry, Central Michigan University, Mt. Pleasant, MI 48859, D. A. Yphantis and T. Laue*, Biochemistry and Biophysics Section, Life Sciences Division, University of Connecticut, Storrs, CT 06268.

Precise, high resolution manual measurement of photographs is an extremely laborious and time consuming procedure. Numerous automated mechanical densitometers have been described, but most are too slow to be practical for the high resolution measurement of large numbers of photographs. High speed television (TV) based densitometers have also been described. However, when a conventional TV camera is used to convert data from the optical to the electronic domain for subsequent digitization and processing, the data conversion bandwidth required for adequate resolution far exceeds the capabilities of most laboratory data acquisition systems. Two TV-based image measurement systems that significantly reduce the required bandwidth have been constructed. One system incorporates a scan rate converter in the video chain to provide image storage, variable rate readout and random measurement capability. The second system uses a digitally implemented sampling algorithm in conjunction with a mechanical stage to achieve a fixed conversion rate reduction. The construction of both systems and their application to the measurement of Rayleigh interference patterns from the analytical ultracentrifuge will be described. This work was supported by NSF Grants # BMS-71-01299 and PCM 76-21487.

**W-POS-D4** DATA EXTRAPOLATION TO REDUCE RUNNING TIMES IN SEDIMENTATION EQUILIBRIUM EXPERIMENTS John J. Correia. Biochemistry and Biophysics Section, University of Connecticut, Storrs, CT 06268; George H. Weiss, Division of Computer Research, National Institutes of Health, Bethesda, MD 20014 and David A. Yphantis, Biochemistry and Biophysics Section and Materials Science Institute, University of Connecticut, Storrs, CT 06268

It is often desirable to reduce the time required to attain sedimentation equilibrium as, for example, to make feasible studies with unstable systems. One commonly used procedure is overspeeding, which, however, requires previous knowledge of solute parameters and careful control of experimental conditions, and which appears to be of only limited utility with heterogenerous or interacting systems. We propose use of the Aitken transformation to extrapolate data obtained at finite times to equilibrium. According to this transformation the estimated equilibrium concentrations, $C_n(r)$, are given by

$C_n(r) = [C_{n+1}(r)\, C_{n-1}(r) - C_n^2(r)]/[C_{n+1}(r) + C_{n-1}(4) - 2C_n(r)]$ where the $C_n(r)$ are the directly observed concentrations at the radius r and at the times $n\Delta t$. Tests of this procedure with computer generated data demonstrate the theoretical capability of cutting running time from 1/2 to 1/8 the time required for routine sedimentation equilibrium experiments. This possibility is shown to be valid for nonideal and for heterogenous systems as well as for simple ideal systems. A potential drawback to the proposed method is susceptibility to noise. (Supported, in part, by NSF Grants BMS 71-01299 and PCM 76-21487).

**W-POS-D5** COMBINED VELOCITY SEDIMENTATION AND ELECTROPHORETIC LIGHT SCATTERING FOR THE ANALYSIS OF MAMMALIAN CELLS. J. Y. Josefowicz and B. R. Ware, Department of Chemistry, Harvard University, Cambridge, MA 02138, and A. L. Griffith and N. Catsimpoolas, Biophysics Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139

Velocity sedimentation and electrophoretic light scattering have been combined for the first time to analyse mammalian cells. CBA/j mouse thymocytes ( $5 \times 10^7$ cell/ml ) were separated by velocity sedimentation at unit gravity in a 1% to 2% BSA/PBS density gradient at 4°C. Consecutive fractions of the sedimentation distribution were collected, and the cells in each fraction were sized with a Coulter Channelyzer. Electrophoretic light scattering experiments were then performed on the cells in each fraction to determine their electrophoretic mobility distributions. A projection map of sedimentation velocity and electrophoretic mobility can be constructed for each preparation of cells. These maps facilitate visualization of subpopulations of cells defined by the two parameters. Using this method we have distinguished subpopulations of CBA/j mouse thymocytes.
( Supported by NSF Grant No. PCM72-05133, NCI Contract NO1-CB-43928 ( NC ), and a Canada Council Killam Postdoctoral Scholarship ( JYJ )

**W-POS-D6** SIZING OF INSECT VIRUSES BY THE RESISTIVE PULSE TECHNIQUE, ELECTRON MICROSCOPY, AND LASER LIGHT SCATTERING. R. W. DeBlois, D. Cluxton*+, H. Mazzone*++, and E. E. Uzgiris, General Electric Corporate Research and Development, Schenectady, N.Y. 12301.

Polydispersity due to the presence of rod multiplets that are enclosed in membranes is common in some insect virus preparations. This polydispersity renders light scattering fluctuation measurements inaccurate and unreliable as an indicator of virus size or as an indicator of even the extent of the polydispersity. However, quantitative information of the virus multiplet structures is readily obtained in these systems by the resistive pulse technique. Resolved multiplet distributions were obtained for the Gypsy moth virus and N. sertifer virus, and a single size peak was obtained for the Tipula iridescent virus. These measurements were compared with electron microscopy and light scattering determinations of size. The measurements were in substantial agreement for the N. sertifer and the Tipula iridescent viruses but some differences in virus sizes between electron microscopy and the measurements in aqueous solutions were observed for the Gypsy moth virus. Some applications of accurate particle multiplet determinations will be discussed.
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**W-POS-D7** EFFECT OF CELL SHAPE AND ORIENTATION UPON FLOW MICROFLUOROMETRIC ANALYSIS. M.J. Skogen-Hagenson,* Department of Biomedical Engineering, Iowa State University, Ames, Iowa, and J.M. Crowell, Biophysics and Instrumentation Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico.

DNA distributions in a wide variety of cell populations are being studied by flow microfluorometric techniques. For cells lacking symmetry about the flow axis, however, fluorescence is not isotropic and fluorescence measurements are affected by cell orientation. A model is presented for which the angular distribution of fluorescent light is calculated in the case when the flow axis passes through the plane of a flat cell whose orientation about that axis is random. Comparisons of the model with experimental data are shown. The advantages of an ellipsoidal flow chamber are discussed. Multiple detector arrangements are proposed such that orientation effects can be eliminated from the data. (This work is being performed under the auspices of the U. S. Energy Research and Development Administration.)

**W-POS-D8** FAST STOPPED-FLOW THERMAL APPARATUS. R.L. Berger and B. Balko, Laboratory of Technical Development, NHLBI, Bethesda, Maryland 20014.

We have developed a stopped-flow thermal apparatus for the study of fast chemical reactions in solution. Using a data reconstruction scheme it is capable of following first order reactions with $k=2\times10^{3}$ sec$^{-1}$.

This calorimeter is adiabatic to 2% for 2 seconds and returns to equilibrium temperature (95% or better) in less than 2 minutes. It requires 100 μ liters of each solution per reaction. The temperature changes are measured using a hermetically sealed thermistor with a response time $t_{\frac{1}{2}}$ = 2.5 m sec. A specially designed thermistor bridge gives a sensitivity of 100 μ $^{o}$C with a band pass of 140 Hz. Test procedures of the thermal response time are similar to those described earlier (1). The stopped-flow apparatus uses a miniature version of the ball mixer (2) which gives good mixing with a low heat artifact. A finite element analysis simulation technique (FEAST) (3) computer program is used to give a true data reconstruction corrected for both thermal losses and sensor response time thus extending the working range of the instrument. A FEAST program is also used to simulate the chemical reactions under study. We have used the reactions of HCl with NaOH and HCl with $NaHCO_3$, and $CO_2$ with glycyl-glycine to test and characterize the system.

1. B. Balko, R. L. Berger, Rev. Sci. Instr. 39, 498, 1968.
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**W-POS-D9** ANALYSIS FOR ACTINOMYCIN D IN HUMAN SERUM USING MICROENTHALPIMETRY. D. L. Phillips* and D. M. Yourtee, Biochemistry Department, Cancer Research Center, Columbia, Mo. 65201

The utility of actinomycin D, a potent anticancer agent, has been limited for pharmakinetic study by the inability to monitor serum levels of the drug in animals and in patients receiving therapy. We have conducted studies utilizing a microenthalpimeter of the Tian-Calvet (pseudoisothermal) type. This specially-constructed instrument was modified to afford considerably greater sensitivity than previously attainable, allowing assay of actinomycin D to $10^{-10}$ M in serum, by measurement of the enthalpy of binding to 2'-deoxyguanosine. Sera from a number of human donors were investigated to evaluate interference effects. Data obtained suggest that a reproducible assay could be developed. Current results of these investigations will be presented.

**W-POS-D10** EQUILIBRIUM ISOTOPIC EXCHANGE KINETIC PROBES OF ENZYME REGULATORY MECHANISMS: MODEL STUDIES. F. C. Wedler, Biochemistry Program, Dept. of Chemistry, Rensselaer Polytechnic Inst., Troy, New York 12181.

Specific effects of bound modifier (activator or inhibitor) on an enzyme-catalyzed reaction can be distinguished with considerable insight by use of equilibrium isotopic exchange kinetics, that allow one to observe both rate-limiting and non-rate-limiting steps in the overall reaction. Model patterns were obtained for each separate modifier effect considered, using computer-assisted plotting of each effect for a two-reactant, two-product system: A + B = P + Q. Once one has determined substrate binding order (random or compulsory) one must vary each combination of substrate-product pairs in the presence (and absence) of modifier; then, holding substrate concentrations constant at ca. 2 x $K_m$, vary the modifier levels. In each case, one observes the exchange rates R (A $\rightleftarrows$ P) and R' (B $\rightleftarrows$ Q) as a function of [substrate] or [modifier]. Changes in $K_m$ (app) or on-off rates are easily recognizable, especially in double-reciprocal plots. Unique sets of kinetic response patterns are obtained for each separate modifier-induced change in rate of the catalytic interconversion step, substrate association, substrate dissociation or substrate association-dissociation. Such model derivations, using best-estimate values of rate constants, have proven very useful in understanding mechanisms of certain key regulatory enzymes. (Supported by NSF Grant PCM-76-22340).

**W-POS-E1** SPECIFIC KERR CONSTANTS OF RIGID, ELLIPSOIDAL MACROMOLECULES IN CONDUCTING SOLUTION AT VERY LOW IONIC STRENGTH. S. Krause and M. E. Galvin,* Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N.Y. 12181

The theoretical treatment of the Kerr Constant of rigid, conducting, dipolar macromolecules of O'Konski and Krause (1970), J. Phys. Chem., 74, 3243, has been extended to very low ionic strength solutions. The O'Konski and Krause theoretical treatment postulated a surface conductivity, directly along the surface of each macromolecule, caused by movement of the counterions of the macromolecule. This surface conductivity, considered in relation to the bulk conductivity of the solvent, was used to calculate the free energy of the macromolecules in an electric field. This theoretical treatment qualitatively predicts the decrease in Kerr Constant with increasing ionic strength found for a nonpolar strain of TMV by O'Konski and Haltner (1957), J. Amer. Chem. Soc., 79, 5634, but it also predicts an increase of Kerr Constant with increasing ionic strength for dipolar macromolecules. This is not in accord with experimental data. In the present work, it has been assumed that, at very low ionic strength, the average counterion is at the Debye characteristic distance from the surface of each macromolecule and contributes to the surface conductivity of the macromolecule at that distance. This makes it possible to predict a decrease in Kerr Constant for dipolar macromolecules with increasing ionic strength at very low ionic strength, followed by an increase in Kerr Constant at slightly higher ionic strength, followed, finally, by a levelling off of the Kerr Constant to a constant value at high ionic strength. The new theoretical predictions will be compared with experimental data on some proteins and bacteriophages.

**W-POS-E2** DEPENDENCE OF ELECTRICAL BIREFRINGENCE DECAY OF LENGTH POLYDISPERSE MACROMOLECULES ON THE ORIENTING ELECTRICAL FIELD STRENGTH. M.M. Judy and S.A. Bernfeld*, Biophysics Department, University of Texas Health Science Center, Dallas, Texas 75235.

Decay of the electrical birefringence of long rigid macromolecules depends on their length and contains information about molecular length distribution. For a continuous length distribution, the birefringence decay $\Delta n(t)$ normalized to the steady state value $\Delta n_o$ is

$$\Delta n(t)/\Delta n_o = \int \Delta n_o(\ell)\exp[-6D(\ell)t]d\ell / \int \Delta n_o(\ell)d\ell \qquad (1)$$

where integration is over molecular length, $D(\ell)$ is the rotational diffusion constant of a molecule of length $\ell$. $\Delta n_o(\ell)$ is the contribution to the birefringence of molecules having lengths between $\ell$ and $\ell+d\ell$ and is proportional to the volume fraction of molecules in the length range. $\Delta n_o(\ell)$ also depends on the molecular electrical dipole and the magnitude of the orienting field. In previous studies[1] the first through third time derivatives of equation (1) at small electrical fields where $\Delta n_o$ depends on $E^2$ were derived and shown to depend uniquely on the parameters a,b,s which characterize the length distribution $h(\ell) = \ell^s \exp[(\ell-a)/b]$. For $s = o$, $h(\ell)$ describes the equilibrium length distribution of helical and linear polymers and for $s \neq o$ the meta-stable distribution formed by addition of monomer to pre-existing nuclei. We report here the derivation of equations for the n-th time derivative of equation (1) for decay from orientation in electrical fields of arbitrary strength. These equations allow characterization of molecular length distributions from decay data obtained at large field strengths where signal to noise ratio is enhanced.

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**W-POS-E3** MOLECULAR MECHANISM OF THE LIPID DEPOSITION IN ATHEROSCLEROSIS: A PLAUSIBLE HYPOTHESIS. T. Kirchhausen and L. Mateu.* Laboratorio Estructura Molecular, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 1827, Caracas-101, Venezuela.

Atherosclerosis is a disease characterized by the thickening of the major arteries in response to the deposition of lipids, mainly from low density lipoproteins (LDL). Even though the lipid composition of the intact LDL particles and the extracellular lipids extracted from advanced atherosclerotic lesions is very similar (E. Smith and B. Slater, in Atherogenesis, Ciba Foundation, page 40, 1973), the physical state of them is extremely different, namely, at body temperature, the state of the lipids in LDL is liquid isotropic while in the lesions these molecules are organized in "solid" phases.

Using small-angle X-ray techniques we have explored the thermal behaviour of intact and denatured LDL and compared them with lipid model systems. Our results are discussed in terms of a mechanism in which the interaction of phospholipids with cholesterol esters (as a consequence of the disruption of LDL in the arteries)would be the main responsible for the precipitation of lipids in the intima.

W-POS-E4 SODIUM-23 NMR STUDIES OF CATION-DNA INTERACTIONS, C. F. Anderson*, P. A. Hart*, and M. T. Record, Jr., Department of Chemistry, University of Wisconsin, Madison, Wisconsin, 53706.

Sodium-23 NMR has been used to study the nature and extent of the association of monovalent cations with double-stranded DNA in aqueous solution. It is known that the translational diffusion of $Na^+$ in polyelectrolyte solutions is sufficiently rapid that the linewidth of the sodium peak is a population-weighted average of the relaxation rates in all accessible environments. Hence, $^{23}Na$ NMR affords a direct probe of the microscopic distribution of the cations. As a quaternary ammonium salt of DNA is titrated with NaCl, the variation in the $^{23}Na$ linewidth can be explained by means of Manning's concept of condensation.[1] In agreement with earlier work[2] no detectable fraction of $Na^+$ appears to be rigidly site-bound, and the mean relaxation rate of the associated ions is less than ten times faster than that of ions free in solution. Essentially all the sodium is associated below a definite value of $[Na^+]/[\text{DNA monomers}]$, (.85±.05 for the tetrabutylammonium salt.) Above that value, the linewidth is linear with $1/[Na^+]$, indicating that no additional ions become associated as their total concentration is increased. Further studies are in progress to test the effect of varying concentrations of other monovalent and divalent cations on the association of sodium with DNA.

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W-POS-E5 PROBLEMS RELATED TO ACTIVE SITE HETEROGENEITY IN HIGH POTENTIAL IRON/SULFUR PROTEINS. J.A. Fee, M.S. Goldsmith, J. Hejna, J. Lee, B. Antanaitis, J. Reid, W.R. Dunham, and R.H. Sands, Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109

In a spectroscopic study of the four iron/sulfur clusters of high potential iron proteins (HiPIP) from several photosynthetic bacteria we have edicited evidence for a microscopic heterogenity of unknown origin. This heterogeneity is exhibited in the EPR and Mossbauer spectra of HiPIPs from C. vinosum D, R. gelatinosa and R. tenue. The EPR spectrum of the oxidized HiPIP from C. vinosum exhibits three distinctly different signals when the protein is in 0.2M NaCl or various concentrations of ethanol. The EPR spectral simulations will be displayed and the individual component spectra will be characterized. Similar decompositions of the EPR spectra from the oxidized HiPIPs of R. gelatinosa and R. tenue also will be displayed. The Mossbauer spectra of the oxidized states of these proteins also exhibit the heterogeneity, although in these cases we do not see any salt effect on the spectra and only two distinct spectra are discernable. The second specie exhibits no hyperfine interaction and represents from 7 to 16% of the total iron depending on the particular protein. These measured ratios match the percentages recorded in the EPR spectra for one of the component signals seen there. Possible models for the nature of the heterogeneities will be described.

W-POS-E6 QUANTITATION AND CHARACTERIZATION OF THE TRIFLUOROACETONYL GROUP; A USEFUL NMR PROBE. W. E. Brown* and K. B. Seamon*, (Intr. by R. V. Rice), Departments of Biological Sciences and Chemistry, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213.

Bromotrifluoroacetone has been shown to be a sulfhydryl-specific reagent for proteins and peptides and as such a useful probe in protein NMR studies. The present study describes methods of quantitating the extent of sulfhydryl modification by BrTFA and verifies the proposed products by NMR. As a model, the cysteine of glutathione was alkylated and the 250 MHz proton NMR spectrum was consistent with the expected structure. The trifluoroacetonyl derivative was reduced with sodium borohydride. The site of reduction was verified by NMR to be the carbonyl moiety of the trifluoroacetonyl group. The methods for quantitating the trifluoroacetonyl derivative are based on the observed stability of the reduction product to both oxidation and acid hydrolysis. Two quantitation methods are presented. First, in peptides containing no threonine, the reduced, alkylated peptide is acid hydrolyzed and the reduced trifluoroacetonyl cysteine thus produced is directly quantitated as a distinct peak appearing at a position two minutes before but not resolved from threonine on the long column of a Beckman amino acid analyzer. Alternatively, in peptides containing threonine, the reduced, alkylated peptide is acid hydrolyzed in the presence of DMSO and the unmodified cysteine quantitated as cysteic acid. The difference *in cysteic acid* content between samples of unalkylated and alkylated peptide is therefore equal to the quantity of alkylated cysteine in the modified peptide. These procedures have been successfully applied to alkylated proteins. (This research was supported in part by a Grant from the Winters Foundation and NIH Grant RR00292.)

W-POS-E7 PHOSPHOLIPID-PROTEIN INTERACTIONS IN HUMAN LOW DENSITY LIPOPROTEIN. P.L.Yeagle*, R.B. Martin*, R.G. Langdon, Departments of Chemistry and Biochemistry, University of Virginia, Charlottesville, Virginia 22901.

$^{31}P$ nuclear magnetic resonance spectra of human low density lipoprotein (LDL) have been obtained and the major phospholipid components identified. Analysis of the spectra of intact LDL revealed two phospholipid environments; one occupied by 4/5 of the phospholipid with high resolution resonances and properties similar to phospholipids in vesicles, and a second occupied by 1/5 of the phospholipid with broad lines indicative of immobilization. Limited tryspin treatment of the particle cleaved all of the B peptide into smaller molecular weight peptides which remained with the particle. Trypsin-treated LDL eluted from a Sepharose CL-6B column similarly to native LDL, so that the modified particle remained intact. $^{31}P$ spectra of trypsin-treated LDL showed little or no immobilization of phospholipids. The immobilization in the native LDL particle has been attributed to lipid-protein interactions between 1/5 of the phospholipid and the B peptide. No analogous lipid-protein interactions were observed with human high density lipoprotein. (This work was supported by an NIH postdoctoral fellowship to PLY, an NSF grant to RBM, and an NIH grant to RGL.)

W-POS-E8 THE SOLUTION PROPERTIES OF CONNECTIVE TISSUE POLYSACCHARIDES. M. E. McDonnell and A. M. Jamieson, Department of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106.

A combination of diffusion and viscosity measurements has been demonstrated to be an effective probe of molecular weight, degree of branching, and unperturbed hydrodynamic dimensions of polymer species (M. E. McDonnell and A. M. Jamieson, Biopolymers 15, 1283(1976)). These procedures are used here to characterize selected mammalian connective tissue polysaccharides. Quasielastically scattered light is analyzed to give a rapid, accurate measurement of the diffusion coefficient as a function of polysaccharide concentration, salt molarity, and temperature. Conventional capillary viscometry methods are also employed. New techniques are developed to deal with the high charge density in chondroitin sulfate by extrapolating measurements to infinite salt concentration. The high degree of aggregation observed if samples are not treated with immense care suggests that some polysaccharide values in the literature may be characterizations of aggregate rather than individual molecules. Samples of hyaluronic acid with different molecular weights are analyzed to investigate the applicability of various hydrodynamic models. This work was supported by USPHS under grant number AM-17110-09.

W-POS-E9 THE STUDIES OF SPERM-CERVICAL MUCUS INTERACTION BY LASER LIGHT-SCATTERING. W.I. Lee, P. Verdugo, R.J. Blandau* and P. Gaddum-Rosse*. Center for Bioengineering and Department of Biological Structure, University of Washington RF-52, Seattle, WA 98195.

The effect of sperm penetration on the molecular structure of cervical mucus was studied in vitro by laser light-scattering spectroscopy. Cervical mucus obtained from cows in midcycle was placed inside a flat demountable glass cell in juxtaposition to washed bull spermatozoa obtained from freshly ejaculated semen. Measurements were carried out on the polarized scattered light as a function of scattering angle. The autocorrelation function of the scattered light has the form of an exponential decay with more than one decay time constant. Results indicate that sperm penetration produces a disruption in the molecular structure of the cervical mucus that is inhomogenous and is characterized by a decrease in the decay time constant of the autocorrelation function. However, the exhibition of a long time constant in the autocorrelation function, particularily at low scattering angles, suggests a coupling between rotational motion of non-spherical mucin segments and a severe congestion-induced anisotropy of translational diffusion.

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**W-POS-E10** CIRCULAR DICHROISM OF RETINAL SCHIFF BASE COMPLEXES. D.D. Muccio, J.E. Draheim, and J.Y. Cassim, Department of Biophysics, The Ohio State University, Columbus, Ohio 43210.

Natural occurring retinal Schiff base complexes are formed in visual systems and certain photosensitive membranes. Circular dichroic studies of such complexes, in situ, have been made in an attempt to elucidate the structure-function relationship of such systems. However the complexity of these systems have made the analysis of such spectra formidable. In an attempt to alleviate this problem we have studied circular dichroic spectra of Schiff base complexes of all0trans retinal with poly-L-lysine and D- and L-lysine. The absorption maximum of the non-protonated form of the poly-L-lysine retinal Schiff base complex is ca 340 nm. Upon protonation the absorption maximum shifts to ca 400 nm. Circular dichroic spectra of the protonated specie shows a pair of oppositely signed bands with nearly equal rotational strengths at ca 380 nm and ca 435 nm with the crossover point centered near the absorption maximum. Similar spectral behavior has been observed also for the D- and L-lysine retinal complexes. Studies of the effects of peptide: retinal ratio on the spectra indicate band positions to be essentially independent of this ratio whereas the rotatory strengths of the positive and negative circular dichroic bands are directly proportional to the number of Schiff bases formed. Of the three mechanisms that can be proposed to explain these results: molecular exciton interactions between like chromophores, different chromophore environments, and nearly degenerate chromophore transitions with opposite ellipticity, the experimental results are in best accord with the last. It is of interest that the visible absorption and circular dichroic spectra of the purple membrane from two halophilic bacteria also show the same spectral relationship as observed for these retinal Schiff base complexes.

**W-POS-E11** MIGRATION OF HETEROGENIOUS DNA IN LINEAR SUCROSE GRADIENTS. B.M.Dancis and J.Gunn*, Department of Biology, Temple University, Philadelphia, Pa. 19122.

The sizes of HeLa DNA samples sheared in a Virtis homogenizer were determined by electron microscopy, agarose gel electrophoresis and sucrose sedimentation. Based on the electron micrographs and gel electrophoresis the standard deviation of the sizes of the DNA fragments in a sample was equal to approximately one half of the mean length and the weight average length was 20-50% greater than the number average length. The DNA samples were migrated through 5-20% linear neutral (pH=8) and alkaline (pH=13) sucrose gradients and the length of DNA in the peak fraction was determined using a phage standard and the relationships of Studier (J.Mol.Biol.11,373,1966). Even though DNA has different sedimentation coefficients in alkaline and neutral solutions, the peak fraction of a given DNA sample was always the same in the two sucrose gradients. In an alkaline gradient the estimated value of the peak was a function of the weight average while in a neutral gradient it was a function of the number average. Since migration in a sucrose gradient is proportional to the log of the fragment length and since the DNA formed a normal distribution, a measurement of the DNA based on amount rather than number should yield a peak at the weight average and should be skewed towards the smaller fragments. These effects are observed in agarose gels but not in neutral sucrose gradients. For DNA fragments between 3000 and 50,000 bases long, sucrose sedimentation is an easier and quicker method for size determination than electrophoresis and is much easier than electron microscopy. In addition many experiments require an estimate of the number average rather than the weight average length. This value can be directly determined from the peak in a neutral sucrose gradient but only after laborious calculation from the entire distribution in an agarose gel. Finally unnicked DNA will show the same migration pattern in the two gradients.

**W-POS-E12** INTERPRETATION OF MAMMALIAN DNA REPAIR STUDIES PERFORMED ON ALKALINE SUCROSE GRADIENTS. K.T. Wheeler, J.D. Linn*, E.S. Chase*, and C.T. Morton*, Brain Tumor Research Center, University of California, San Francisco, CA 94143.

Previous alkaline sucrose gradient studies on the repair of x-ray-induced DNA damage in mammalian cells indicated that the transition from DNA molecules with sedimentation coefficients $\leq$ 165 S to DNA species > 165 S was associated with the maintenance of their reproductive or functional integrity. However, the molecular interpretation of this transition was uncertain. To provide experimental evidence for an interpretation of this transition, we have compared: 1) the dependence of the sedimentation coefficients of these DNA species on rotor speed and radius, 2) the ability of these DNA species to renature, and 3) the ability of free radical scavengers to protect these DNA species against x-ray-induced damage. In general: 1) DNA species with sedimentation coefficients > 165 S do not exhibit a dependence on rotor speed and radius as predicted by Zimm while DNA species $\leq$ 165 S do, 2) DNA species > 165 S renature rapidly even under adverse conditions while species $\leq$ 165 S do not, and 3) free radical scavengers equally protect both sets of DNA species against x-ray damage. Thus, the data support the hypothesis that the transition from DNA species $\leq$ 165 S to DNA species > 165 S represents a conformation change from single-stranded random coil polymers to predominently single-stranded nonrandom coil polymers. (Supported by CA-15203, CA-15325, and *an RCDA to K.T. Wheeler NS70739*)

W-POS-E13 STRUCTURE OF NUCLEIC ACIDS AND OF NUCLEOPROTEINS AS REVEALED BY SEDIMENTATION IN NEUTRAL AQUEOUS TRICHLOROACETATE SOLUTIONS. R.L. Burke, Dept. of Microbiology, State University of New York, Stony Brook, N.Y. 11794. (Research supported by USPH Grant 21176).

The alkali metal salts of trichloroacetic acid (TCA) are generally useful for sedimentation analysis of DNA, RNA, proteins, and nucleoprotein complexes under both native and neutral denaturing conditions at room temperature. NaTCA is best suited for sedimentation velocity studies, RbTCA is most suitable for buoyant banding of native or denatured DNA and CsTCA is the salt of choice for buoyant experiments with either duplex or single stranded RNAs. For DNA in NaTCA, $dT_M/dM = 31.83°CM^{-1}L^{-1}$ (range 2-4M) and in RbTCA $dT_M/dM = 26.04°CM^{-1}L^{-1}$ (range 2-4M). Nucleic acids from intact polio, Adeno2, SV40, T7, λ, and vaccinia virions have been isolated by buoyant banding in RbTCA or CsTCA and examined for the presence of bound protein. In the case of polio virus, a specific protein remains bound strongly to virion RNA, possibly in covalent association. No protein was detected in the DNA bands from T7, λ, or vaccinia viruses. A very large buoyant density increase, 174 mg/ml for nicked circular PM-2 DNA, accompanies the helix-coil transition in RbTCA. This increase permits the partial separation of substantially duplex PM-2 DNAs containing as little as 1% unpaired bases. The complete early melting transition for a family of covalently closed PM-2 DNAs was revealed by the accompanying buoyant density increases in buoyant RbTCA. The separation between nicked and closed circular PM-2 DNAs increases with temperature up to that (35.5°C) at which nicked PM-2 DNA cooperatively melts and subsequently pellets. The great usefulness of the alkali salts of TCA arises from their large denaturing potency, from the large buoyant resolution between native and denatured nucleic acids, and from their utility in the detection of strongly bound proteins.

W-POS-E14 FURTHER CHARACTERIZATION OF T4 ENDONUCLEASE V BY ELECTROPHORETIC ANALYSIS OF SUPERHELICAL DNA ON AGAROSE GELS. P.C. Seawell*, E.C. Friedberg and A.K. Ganesan, Department of Biological Sciences and Department of Pathology, Stanford University, Stanford, California 94305

Supercoils, relaxed circles and double strand linear forms of DNA can be separated into distinct bands by electrophoresis through agarose gels. The bands can be visualized by staining with ethidium bromide and quantified by using radioactive DNA. This technique provides a rapid and sensitive assay for endonucleases capable of producing either single or double strand breaks in superhelical DNA. We have used this assay for the further characterization of T4 endonuclease V, an enzyme that produces single strand nicks adjacent to pyrimidine dimers. Superhelical DNA from SV40 and colE1 was irradiated with UV (principally 254 nm) and used as substrate. After nuclease treatment the average number of single strand nicks per molecule produced by the enzyme was calculated from the fraction of molecules containing no breaks, assuming a Poisson distribution of target sites. For DNA irradiated with UV fluences up to 20 $J/m^2$ the conversion of supercoils to relaxed circles by T4 endonuclease V corresponded to approximately one single strand nick per dimer. After endonuclease treatment of DNA irradiated with higher fluences linear molecules could be detected in amounts proportional to UV fluence. The linear molecules might result from 1) closely spaced incisions in opposite strands due to coincidence of randomly distributed dimers, 2) infrequent double strand cuts by the T4 endonuclease V at the site of a dimer, or 3) double strand cuts at the site of relatively rare photoproducts (perhaps by a nuclease other than the T4 enzyme).

W-POS-E15 CIRCULAR DICHROISM LIGHT SCATTERING CORRECTION BY THE USE OF FLUORESCENCE DETECTED CIRCULAR DICHROISM (FDCD). C. Reich* and M.F. Maestre. Space Sciences Laboratory, University of California, Berkeley, CA 94720.

FDCD is a measure of circular dichroism which detects only those signals produced by the fluorescent chromophores in optically active compounds (1). By the use of a non-optically active fluorescent chromophore that does not interact chemically with a given material it is possible to correct for those perturbations produced by assymetric scattering of large optically active particle such as bacteriophages. This CD spectra together with the absorbance spectra of the solution (e.g. phage plus solution of non-optically active fluorescent chromophore) will provide a CD spectra corrected for scattering perturbations. Preliminary measurement of T2 bacteriophage CD spectra shows agreement with previously obtained CD spectra corrected for scattering by Fluorscat methods and large acceptance angle photomultiplier methods (2). Similar measurements are being done on other bacteriophages such as T5, T7, etc.

(1) D.H. Turner, I. Tinoco, Jr., and M.F. Maestre, Biochemistry (1975) 14, 3794.
(2) B.P. Dorman and M.F. Maestre, Proc. Natl. Acad. Sci. U.S.A. (1973) 70, 225.

**W-POS-E16** PHYSICO-CHEMICAL MEASUREMENTS RELATED TO THE STRUCTURE AND ASSEMBLY OF FILAMENTOUS VIRUSES. R.L. Wiseman, F.C. Chen and L.A. Day. The Public Health Research Institute of the City of New York, New York, N.Y. (10016).

A variety of measurements show that the arrangements of single-stranded circular DNA and protein subunits in the bacterial viruses Pf1, Xf and fd, and the complex between fd DNA and gene V protein isolated from fd infected cells are all different. Key parameters for each filamentous structure are the number of nucleotides, length, DNA content and subunit mass. A new value for the density increment of fd DNA in SSC gives more than 6200 nucleotides by sedimentation equilibrium and sedimentation diffusion. Relative DNA masses based on alkaline sedimentation behaviour of $\phi$x174 DNA are: fd DNA, 1.19; Pf1 DNA, 1.39; and Xf DNA, 1.38. The DNA contents give the ratio of nucleotides to subunits in the four structures: Pf1, 1.0 (corrected value); Xf, 2.0-2.1; fd, 2.32; gene V complex, 4.70. These values together with length determinations by electron microscopy and transient electric birefringence give axial separations between neighboring nucleotides of nearly 6 Å for Pf1, but slightly less than 3 Å for Xf and fd and slightly greater than 3 Å for the gene V complex. The average axial subunit spacing for both Pf1 and Xf is 2.9 Å, but for fd it is 3.4 Å. In addition, we will present spectral data (CD, absorbance and fluorescence) and the results of titrations with NaOH and with N-bromosuccinimide, a tryptophan specific reagent. According to CD, the overall protein conformations of Pf1, Xf and fd are all different. The apparent complete quenching of fluorescence of one of two tyrosines in Pf1 indicates intimate association with DNA, similar to earlier results for the gene V protein complex. NBS titrations show that the tryptophans of fd and Xf, one per subunit, are highly accessible.

**W-POS-E17** NON-SPECIFIC INTERACTIONS OF LAC REPRESSOR AND OLIGOLYSINES WITH DNA, AS STUDIED BY DIFFERENTIAL BOUNDARY SEDIMENTATION VELOCITY, T. M. Lohman, Department of Chemistry, University of Wisconsin, Madison, Wisconsin, 53706.

We have been studying the interactions between proteins and nucleic acids in an effort to understand the sources of stability in these non-covalent complexes. Previously, we have shown that the major contribution to the free energy of non-specific binding is due to the increase in entropy upon formation of the protein-nucleic acid complex, as a result of the concomitant release of counterions previously bound to the DNA and protein. The dependence of observed equilibrium binding constants ($K_{obs}$) on [NaCl] can be used to obtain the maximum number of ionic interactions, m', which are formed in the protein-DNA complex. In order to test the validity of our interpretation of the dependence of $K_{obs}$ on [NaCl], a study of the binding of homogeneous oligolysines to DNA has been carried out, using a differential sedimentation technique. These studies confirm that the binding constants to DNA do vary linearly with [NaCl] as we have predicted, and the calculated number of ionic interactions, m', is equal to the number of charged residues on the oligolysine. This will be useful in dissecting the free energy of protein-nucleic acid interactions into its electrostatic and non-electrostatic components, thereby lending some insight into specificity. The binding interaction of E. coli lac repressor protein with non-specific DNA has also been studied. $K_{obs}$ have been determined as a function of [NaCl] and pH. For this system, we obtain $m'=13\pm1$ as a maximum value for the number of ionic pairs formed in the equilibrium complex with non-specific DNA. (Supported by NSF grant # PCM76-11016 to M. T. Record, Jr.)

**W-POS-E18** LAC REPRESSOR-DNA INTERACTION AS STUDIED BY QUANTITATIVE DNA-CELLULOSE CHROMATOGRAPHY, Pieter L. deHaseth, Department of Chemistry, University of Wisconsin, Madison, Wisconsin, 53706.

We have investigated the influence of environmental variables on the non-specific interaction of lac repressor (R) with DNA (D) in order to better define the thermodynamic parameters governing this non-covalent interaction. We have employed a novel technique, quantitative DNA cellulose chromatography, and have provided the mathematical framework for the analysis of elution profiles in terms of the association constant of the R-D interaction, K(R-D) (Anderson, in preparation). The useful range of the technique is $10^4 M^{-1} < K(RD) < 10^6 M^{-1}$. We find that K(RD) is very sensitive to salt concentration, nature of both anion and cation, pH and temperature. Where a comparison is possible, agreement with the results of Revzin and Von Hippel (personal communication) is good. Analysis of the data indicates the binding of doubly protonated repressor, forming 12±1 ion pairs (Record et al., 1976, JMB, in press) in the complex. The presence of divalent cations (e.g., $Mg^{++}$) reduces the absolute values of K(RD), and also their sensitivity to the concentration of monovalent salts (e.g., NaCl). Comparing our results with those of Riggs et al. (1970, JMB, 48, 67) on the repressor-operator interaction we conclude that the non-specific interaction involves the formation of 1.5 times as many ion pairs as the specific interaction. Our results point to the importance of knowing the ionic environment of the DNA in the cell for an understanding at the molecular level of the functioning of genetic control mechanisms. (Supported by NSF grant #PCM76-11016 to M. T. Record, Jr.)

**W-POS-E19** ARCHITECTURE OF PHOSPHORYLASE *a* AT 3 Å RESOLUTION. J. Sygusch*, N.B. Madsen*, and R.J. Fletterick, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Glycogen phosphorylase *a* from rabbit skeletal muscle has been crystallized from a glucose medium to stabilize the dimeric form. The crystalline protein is enzymatically active and shows normal solution kinetics. A model of the polypeptide backbone of the 188,000 dalton dimer of glycogen phosphorylase *a* has been built from a MIR 3 Å resolution electron density map. Each identical subunit of the dimer has a compact shape with overall dimensions of 85 Å by 75 Å by 55 Å and is tightly associated with its 2 fold symmetry related subunit. There are three major excursions of the polypeptide chain of one monomer across the two fold axis to make extensive contacts with the other subunit.

The two active sites are each shared between the two subunits at their interface. Here are found the binding sites for the substrates, G1P and arsenate, a competitive inhibitor UDPG and allosteric effector AMP. The phosphate moiety of these substrates all bind to the same amino acid residue, which we have shown to be an arginine. The allosteric effector AMP shows a different interaction with the enzyme in a simple binary complex with phosphorylase than it does in the presence of the two substrates G1P and maltoheptaose. Maltoheptaose, a glycogen analogue substrate binds 25 Å (at closest approach) from the glucose-1-P binding site. The possible roles of this site are under investigation. A third region far from the active site has the same topological structure as has been observed for the nucleotide binding domains in the dehydrogenases. Adenine or adenosine (but not AMP) bind here in a position similar to the adenine ring of NAD in the dehydrogenases while glucose and UDPG bind 17 Å away in an interior crevice near the center of the monomer.

**W-POS-E20** EFFECTS OF N-ETHYLMALEIMIDE (NEM) ON CONFORMATIONALLY DEPENDENT TRYPSINOLYSIS OF $(Na^+ + K^+)$-ATPase. T.N. Lo* and E.O. Titus, National Heart, Lung, and Blood Institute, Bethesda, Md. 20014

$(Na^+ + K^+)$-ATPase is a lipoprotein complex containing two polypeptides of approximately 100,000 and 55,000 daltons. Of these, only the larger, which bears the catalytic center, is split when highly purified intact enzyme complex from rabbit kidney outer medulla is incubated briefly with trypsin. Since the pattern of larger fragments obtained can be varied by inclusion of different ligands (Jørgenson, Biochim. Biophys. Acta 401: 399-415 (1975)), the method offers promise for localization of covalently bound, isotopically labeled inhibitors such as $^3$H-NEM. Gel electrophoresis of the products in SDS revealed that in the presence of $Na^+$ or ATP trypsinolysis yields a protein of 75,000 daltons plus other smaller peptides. This protein was no longer predominant when $Na^+K^+$-ATPase had had prior incubation for 15 min in 5 mM NEM, followed by washing with 1% mercaptoethanol. The distribution of metabolites now more nearly resembled that obtained by trypsinolysis of untreated enzyme in the presence of $K^+$, in which a peptide of 43,000 daltons was a major product. Prior reaction with NEM increased the yield of smaller fragments (37,500-39,000) in hydrolyses carried out with $K^+$. Cleavage with trypsin thus promises to be useful in localizing SH groups blocked by NEM, but labeling with NEM precludes the use of ion- and ATP-dependent conformational change to control the point of attack by trypsin.

**W-POS-E21** CIRCULAR DICHROISM OF E. COLI UDP-GALACTOSE-4-EPIMERASE. S.S. Wong, P.A. Frey* and J.Y. Cassim, Depts. of Chem. and Biophys., The Ohio State Univ., Columbus, Ohio 43210

The circular dichroic spectra of UDP-galactose-4-epimerase from E. coli in its reduced (inactive) and oxidized (active) states were studied in order (1) to characterize the secondary structure of the protein, (2) to determine if any significant conformation differences exist between the inactive and active states of the protein and (3) to obtain information concerning the immobility of the bound coenzyme. The far-UV spectra (190-260 nm) of both states of the protein are similar with a negative band ca 220 nm, a negative shoulder ca 208 and a positive band ca 194 nm. However, the ellipticities of the inactive state are minimally 7% greater than those of the oxidized state. The near-UV spectra (260-400 nm) of the reduced state shows a strong positive band at 340 nm, a less intense positive band at 296 nm and a negative band at 280 nm; whereas, the active state shows a very weak positive band at 340 nm and negative bands at 295 and 280 nm. The oscillatory and rotational strengths of the near Gaussian positive band at 340 nm of the reduced protein attributed to a $\pi$-$\pi^*$ transition of the coenzyme, DPNH, are 0.142 and $4.75 \times 10^{-39}$ erg cm$^{-3}$, respectively. On the other hand, in the free state of the coenzyme, this transition has a oscillatory and rotational strength of 0.128 and $1.08 \times 10^{-40}$ erg cm$^{-3}$, respectively. These results suggest that (1) the secondary structure of the protein can be characterized as a combination of the $\alpha$-helical, $\beta$ and unordered conformations (2) reduction of the protein results in a small but reproducible secondary structure change probably due to an increase in $\alpha$-helical content at the expense of unordered form and (3) in view of the 44 fold increase in rotational strength with no appreciable change in oscillatory strength of the 340 nm band of the reduced state, the coenzyme is relatively well immobilized in the protein

**W-POS-E22** PHOSPHORUS-31 NMR STUDIES OF THE INTERACTION OF PYRUVATE KINASE-NUCLEOTIDE COMPLEXES WITH THE MAGNESIUM ION. Raj K. Gupta and Albert S. Mildvan, Institute for Cancer Research, Philadelphia, Pa. 19111

In addition to an enzyme-bound divalent cation, muscle pyruvate kinase (PK) requires a second divalent cation for activity, presumably bound to the nucleotide (Biochemistry 15, 2281). The broadened $^{31}P$ resonances of ATP and ADP bound to PK can be observed at high enzyme levels (>1 mM) and equimolar substrate concentrations. Except for the β resonance of ADP, which is shifted 1 ppm downfield, the enzyme does not affect the chemical shifts. Upon saturation with $Mg^{2+}$ at pH 6.5, the chemical shifts of the α and β resonances of enzyme-bound ADP increase by 0.5 and 1.0 ppm and the β and γ resonances of enzyme-bound ATP increase by 2.0 and 2.4 ppm while the α resonance of ATP increases by 0.5 ppm. These results indicate that on PK, $Mg^{2+}$ coordinates the α and β phosphoryl groups of ADP and the β and γ phosphoryl groups of ATP, suggesting a change in the ligands of $Mg^{2+}$ during phosphoryl transfer. Accordingly, CrATP, a substitution inert tridentate complex of ATP is not a substrate for the phosphoryl transfer reaction of PK although it promotes the enolization of pyruvate (J. Biol. Chem. 251, 2421). No phosphoryl transfer (<0.2% of the CrATP) was detected in the presence of 0.9 mM PK, 1 mM CrATP, 10 mM $Mg^{2+}$, 100 mM $K^{+}$ and 18 mM pyruvate, at pH 6.8. Also no phosphoryl transfer from P-enolpyruvate to α,β CrADP (<0.002% of the rate with ADP) was detected in the presence of 0.2 mM PK. Hence a change in coordination of the nucleotide bound metal is required in the phosphoryl transfer reaction of PK. Oxalate, an analog of the enolate of pyruvate further broadens the $\gamma$-$^{31}P$ resonance of MgATP on PK confirming the van der Waals contact between the enzyme bound substrates (J. Biol. Chem. 251, 2431).

**W-POS-E23** GEPEK: GENERAL PURPOSE COMPUTER LANGUAGE FOR THE ANALYSIS OF ENZYME KINETIC DATA. John A. Russo, Robert A. Mitchell, Dept. of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201

A programming language which may be used to aid the enzyme kineticist in the analysis of data is described. The language is designed to be used in solving a wide range of frequently encountered problems rather than only those having a specialized application. The language proper is relatively simple to use in comparison to other computer languages, but it also embodies the PL/I language and some further extensions of PL/I which makes it an extremely powerful and flexible programming language. Complex data structures that allow efficient I/O operations as well as the manipulation of kinetic data may be constructed by using only a few GEPEK statements. This allows the user to deal with such tasks as equation fitting and statistical analysis of data with a minimum of programming effort.

PL/I extensions include control structures such as the REPEAT UNTIL and SELECT CASE OF constructs. An extensive program library supplements the language and facilitates matrix manipulations and statistical analysis procedures. A function integrator is included for the numerical solution of differential equations using a Runge-Kutta method. Furthermore, various clock, lag time and function generators are available, all of which are useful in the simulation of enzyme mechanisms. A plot command is also provided to allow the display of data by means of a CRT graphics terminal or CALCOMP plotter. The language does not require any special compiler but rather a program is used to translate the source program into a PL/I program which then may be compiled by a standard PL/I (F) compiler. The object deck generated may then be used directly to process kinetic data at a cost substantially less than comparable software interpreters.

**W-POS-E24** POLY(ETHYLENEGLYCOL) IN AQUEOUS SOLUTION: SOLVENT PERTURBATION AND GEL FILTRATION STUDIES. Kenneth C. Ingham, American Red Cross Blood Research Laboratory, Bethesda, Maryland 20014.

As part of an investigation of the mechanism of precipitation of proteins by synthetic polymers, we have measured the ability of oligomers and polymers of ethylene glycol to (a) alter the solubility of amino acids, (b) perturb the absorption spectra of aromatic amino acids, and (c) enhance the fluorescence of 1, 8-anilinonaphthalene sulfonate (ANS). All of these effects increased smoothly with increasing degree of polymerization, up to a molecular weight of about 400. This trend can be partially attributed to the diminishing influence of the terminal -OH groups. However, small analogues such as dioxane and dimethoxyethane (lacking -OH groups) were much less effective than the polymers, suggesting a cooperative effect between neighboring ethoxide residues. On exclusion chromatographic columns, PEG's eluted much earlier than did proteins of comparable molecular weight. This discrepancy diminished in 6M guanidineHCl where the proteins, as random coils, eluted much earlier. By contrast, the elution of PEG was only slightly affected suggesting a random coil configuration even in the absence of denaturant. Carbowax 20 M, a mixture prepared by coupling 2 moles of PEG-6000 with an epoxide, was resolved into two components on Sephadex G-100. The fast-eluting component (the coupled product) was unusually effective in enhancing ANS fluorescence and was shown to bind the dye with $K_a = 5.1 \times 10^{+3} M^{-1}$ at 25°C.

**W-POS-F1** HELICAL PARAMETERS OF THE DNA A AND B FORMS CALCULATED FOR DEOXYDINUCLEOSIDE PHOSPHATES. S. Broyde, B. Hingerty*, R. M. Wartell and S. D. Stellman*, School of Physics, Georgia Institute of Technology, Atlanta, Ga. 30033, and Biology Dept., New York University, N.Y., N.Y. 10003.

Classical potential energy calculations have been made for the deoxydinucleoside phosphates dApdA, dCpdC, dTpdT, dGpdG and dGpdC. In these calculations the energy was minimized with all eight backbone torsional angles and the sugar pucker flexible. The A and the B forms were low energy local minima for all the molecules except dGpdG. These conformations had dihedral angles very similar to those obtained in A and B DNA fibers. We have used these A and B form minimum energy conformations as building blocks for generating larger polymers, and we have calculated their helical parameters. The calculated helices are all right handed. The average vertical distance between the planes of the bases was between 3.2 A and 3.4 A, except for B form dTpdT. However, the helical parameters proved to be very sensitive to small conformational differences. The number of nucleotides per turn ranged from 5 to 9. This was associated with a wide variation in helix radius, from 4.4 A to 8.5 A. In addition the translation per residue along the helix axis is consistently less for the A forms than the B forms. This lower translation per residue is associated with a higher angle of tilt between the normal to the base planes and the helix axis.

**W-POS-F2** NUCLEIC ACID CONFORMATION DETERMINED FROM CARBON-13 NMR HETERONUCLEAR COUPLING CONSTANTS. J.L. Alderfer and P.O.P. Ts'o, Division of Biophysics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md. 21205.

NMR studies on the carbon-13 phosphorous-31 coupling constants provide useful information about the conformation of bonds $\phi$(C5'-O5') and $\phi'$(C3'-O3') of the furanose-phosphate backbone in nucleic acids. The $^3J_{C2',P3'}$, $^3J_{C4',P3'}$, and $^3J_{C4',P5'}$ values were determined in aqueous solution as a function of pH for a series (A,U,C, and T) of 3'-and 5'-mononucleotides having both the ribo- and deoxyribo-furanose. The $^3J_{C4',P5'}$ value of all the 5'-mononucleotides was in the range 8.5-8.9 Hz. However, the 3'-ribo-mononucleotides had unique $^3J_{C2',P3'}$(2.4-4.9) and $^3J_{C4',P3'}$(3.9-6.1) values which showed a large dependence on pH; while the 3'-deoxyribo-mononucleotides had similar $^3J_{C2',P3'}$ (2.9-3.6) and $^3J_{C4',P3'}$ (5.5-6.3) values which only varied slightly over the pH range 4-8. The $^3J_{C2',P3'}$, $^3J_{C4',P3'}$, and $^3J_{C4',P5'}$ were also determined in dinucleoside monophosphates, which had values at 28° of 3.7, 4.9, 9.8 (rAprA), 4.2, 4.9, 8.9 (rUprU), 3.5, 5.8, 9.4 (rCprC), 1.3, 9.1, 9.7 (dApdA), 2.9, 7.5, 9.0 (dTpdT), and 3.2, 6.8, 8.8 (dCpdC), respectively. The observed differences among these monomers and dimers for $^3J_{C4',P5'}$($\Delta$1.2 Hz), $^3J_{C2',P3'}$ ($\Delta$3.6 Hz), and $^3J_{C4',P5'}$($\Delta$5.2 Hz) could represent changes in rotation of bonds $\phi$ and $\phi'$ by $\sim$ 25° and 25-35°, respectively. From the J values it is clear that for dApdA, $\phi$ is predominantly gg and $\phi'$ is mainly gt, conformations which favor base-base overlap, while rUprU shows the least preference for these conformations. The furanose-base conformation of the bond $\chi$ (C1'-N) was also studied using $^3J_{C2,H1'}$, obtained from proton-coupled carbon-13 nmr spectra. Small differences in the $^3J_{C2,H1'}$ value were observed for the uridylic acid series (2.0-2.5 Hz). These changes suggest small variations in the average torsion angle, $\chi$, occur depending on the position of the phosphate (3'- or 5'), pH and solvent. (Supported by NSF and NIH)

**W-POS-F3** HYDRODYNAMIC ANALYSIS OF THE REACTION OF SUPERHELICAL DNA WITH CHEMICAL PROBES: CONSIDERATIONS REGARDING THE REACTIVITY AT UNPAIRED BASES AND THE UNWINDING OF SUPERHELICAL DNA WITH CHEMICAL PROBES. J. Lebowitz, A.K. Chaudhuri*, A. Gonenne*, W. Dean*, and M. Woodworth-Gutai*, Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294 and Dept. of Biology, Syracuse University, Syracuse, N.Y. 13210.

Examination of the secondary structure of superhelical DNA has revealed sensitivity to chemical probes for unpaired bases, single strand specific nucleases and DNA binding proteins. A recent characterization of the reaction of superhelical PM2 DNA I with N-cyclohexyl-N'-$\beta$-(4 methyl morpholinium)ehtyl carbodiimide (CMC) reveals the following: The $S_{20,w}$* increases by five S units upon 1% modification. There is a plateau in $S_{20,w}$* between 1 and 4% reactivity. The extent of reactivity was determined by buoyant density and $^{14}C$ radioactive CMC binding measurements. Further reactivity was not explored since Pulleyblank and Morgan's (1) data of $S_{20,w}$* vs. % reactivity from 6 to 34% was previously published. The results of initial reacitvity are complementary to the cited results of the above authors. It is shown that a model in which superhelical DNA is proposed to contain small intrastrand hairpin regions can be extended to account for the observed transitions in $S_{20,w}$* vs. reactivity for CMC, as well as previous published data for HCHO. A review of the $S_{20,w}$* behavior of various superhelical DNAs upon chemical modification and an extension of the previous work of Upholt, Gray and Vinograd (2) suggests that a general model can be developed to account for the observed behavior of superhelical DNA under a variety of conditions.

(1) Pulleyblank, D.E., and Morgan, A.R. (1975) J. Mol. Biol. 91:1-13.
(2) Upholt, W.B., Gray, H.B.,Jr., and Vinograd, J. (1971) J. Mol. Biol. 62:21-38.

**W-POS-F4** CONFORMATION OF CHICKEN ERYTHROCYTE CHROMATIN MULTIMERS IN LOW IONIC STRENGTH SOLVENT. K. S. Schmitz, Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri 64110 and B. R. Shaw, Department of Chemistry, Duke University, Durham, North Carolina 27706

Chicken erythrocyte chromatin was partially digested by micrococcal nuclease. The hydrodynamic properties of selected multimeric fractions obtained from a Bio-Rad A-5m column were characterized by quasielastic light scattering (diffusion coefficient, D) and sedimentation velocity (Svedberg coefficient, S). These fractions represent a homologous series of a "string-of-beads" molecule, where the "beads" are the nucleosomes (core particle, PS particle, or nu body) in the chromatin microstructure. The molecular weight dependence of $S_{20}$ and $f/f_o$, where f is the molecular friction factor and $f_o$ is the friction factor of an equivalent sphere, suggest: 1) The "spacer region" has a significant contribution to the hydrodynamic properties of the dimer; 2) The power dependence of the molecular weight in the empirical expression

$$S_{20} = 0.011\ M^{.554}$$

suggests the supermolecular structure of the polynucleosomes is compact; 3) Model calculations using the Kirkwood theory for $f/f_o$ eliminates rigid rod, regular polygon, random walk, and closest-packed spherical structures as a possible conformation for the polynucleosomes; and 4) In low ionic strength, either the helix or flexible coil conformations with attractive interactions between nucleosomes is consistent with our data.

**W-POS-F5** UREA EFFECTS ON CHROMATIN NU BODIES. D.E. Olins, P.N. Bryan*, A.L. Olins, Univ. of Tenn.-Oak Ridge Grad. Sch. of Biomed. Sci., Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN. 37830, R.E. Harrington, Dept. of Chem., Univ. Nevada, Reno, NV. 89107, W. E. Hill, Dept. of Chem., Univ. Montana, Missoula, MT. 59801.

Monomer Nu bodies ($\nu_1$) from chicken erythrocyte nuclei consisting of $(H4,H3,H2A,H2B)_2$ associated with ∿140 np of DNA have been exposed to 0-10 M urea, 0.2 mM EDTA, pH 7. Up to, at least, 8 M urea, histones remain associated with DNA. Hydrodynamic studies (S and [η]) indicate a continuous increase in apparent particle volume and/or asymmetry. Measurements of $d\rho/dc$, combined with the other hydrodynamic parameters, permit estimation of β as a function of urea. Electron microscopy on the same samples demonstrates: 0-2 M urea, 80-100 Å dia. spheroid particles; 4-6 M, swollen rings and crescents 200-250 Å dia.; 8-10 M, rod-like structures. Thermal denaturation of $\nu_1$ reveals that for 2/3 of the DNA hyperchromicity ($A_{260}$), $T_m$ decreases linearly with increased urea (i.e., $\Delta T_m/\Delta M$ urea = -2.5°); naked DNA in various NaCl solutions exhibited a similar magnitude of urea destabilization. Circular dichroism (CD) studies of $\nu_1$ displayed a cooperative disappearance of the $\Delta\varepsilon_{222}$ with increased urea (i.e., 50% denaturation at 5 M urea); $\Delta\varepsilon_{280}$ increased continuously. By 8-10 M urea the CD profile of $\nu_1$ was similar to a typical DNA B-like spectrum. As assayed by the binding of N-ethyl maleimide, the -SH group of H3 remains buried until ∿7 M urea. The two domains of a ν body (i.e., the DNA-rich shell, and the protein-rich core) appear to respond differently to the destabilizing effects of increasing urea: DNA conformation and stability exhibits noncooperative effects; the core protein structure reveals cooperative destabilization. Parallel studies of the inner histones (heterotypic tetramer) in 0-10 M urea, 2 M NaCl, pH 7, reveal a cooperative disappearance of $\Delta\varepsilon_{222}$ similar to that observed for $\nu_1$. Research supported jointly by ERDA and grants from NIH and NSF.

**W-POS-F6** LASER-RAMAN SPECTROSCOPY OF CHROMATIN NU BODIES. B. Prescott*, G. J. Thomas, Jr., Dept. of Chem., Southeastern Mass. Univ., North Dartmouth, MA. 02747, D. E. Olins, Univ. of Tenn.-Oak Ridge Grad. Sch. of Biomed. Sci., Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. 37830.

Conformational structures and intermolecular interactions of monomeric Nu bodies ($\nu_1$), from chicken erythrocyte nuclei, have been investigated by laser-Raman spectroscopy. Raman spectra of high signal-to-noise quality and minimal background fluorescence have been obtained for total $\nu_1$, KCl-soluble $\nu_1$ (s-$\nu_1$), KCl-insoluble $\nu_1$ (i-$\nu_1$), DNA, the inner histones "heterotypic tetramer" (H4,H3,H2A,H2B), and erythrocyte chromatin. Small spectral differences between s-$\nu_1$ and i-$\nu_1$ indicate that s-$\nu_1$, is slightly deficient in relative amounts of aliphatic amino acid residues, compared to i-$\nu_1$ or total $\nu_1$. Comparison of the spectral data permit the following conclusions: (1) virtually all of the DNA of $\nu_1$ resembles the "B-genus" structure; (2) the inner histones in $\nu_1$ show a predominantly α-helical structure with little, if any, anti-parallel β pleated sheet. Furthermore, the conformational structure of $\nu_1$, as viewed by laser-Raman spectroscopy, remains essentially unchanged between 0-4 M KCl and 0-50°C. Dissociation of $\nu_1$ by high KCl, therefore, does not markedly perturb these aspects of DNA or histone conformations. A comparison of the Raman spectra of $\nu_1$ and chromatin reveals considerable similarity in DNA and protein conformations. Assignment of Raman lines to vibrations of specific functional groups in DNA and histone, and their sensitivity to DNA-histone interaction will be discussed.

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**W-POS-F7** HIGHER ORDER ULTRASTRUCTURE OF CHROMATIN. Ada L. Olins, Univ. of Tenn.-Oak Ridge Grad. Sch. of Biomed. Sci., Biology Division, Oak Ridge Natl. Laboratory, Oak Ridge, TN. 37830.

The first level of chromatin organization, the ν body, has a unique structure which was substantiated by biochemical, physical, and ultrastructural data. In the present study the persistence of ν bodies in the next higher order of chromatin structure, 250-300 Å fibers (noodles), is demonstrated. Freshly isolated chicken erythrocyte nuclei were swollen in .02 M KCl, 0.5 to 5.0 mM $MgCl_2$, fixed in formaldehyde and spun onto a freshly glowed carbon film. These grids were rinsed in Photo flo, dried and negatively stained with uranyl acetate or uranyl formate. When viewed in the electron microscope, separated chromatin fibers 250-300 Å in diameter were observed streaming from the ruptured erythrocyte nucleus. At high magnification ν bodies could be readily visualized close-packed within the thick fibers. More stretched regions periodically interspersed between the 250-300 Å chromatin fibers, exhibited the typical "beads-on-a-string" morphology. In contrast to the statements of J. T. Finch and A. Klug [PNAS 73, 1897 (1976)] chromatin fibers do not take the appearance of a "continuous density rod...with lack of contrast seen along the nucleofilaments." Rather, the 250-300 Å fibers exhibit clear contrast between close-packed particles. These electron micrographs are consistent with previous observations in our laboratory that monomer ν bodies ($\nu_1$) can give all the low angle x-ray reflections observed for native chromatin, and calculations by R. D. Carlson and D. E. Olins [Nucleic Acid Res. 3, 89 (1976)] that many close-packed arrays of 110 Å spheres can produce these reflections. Research supported jointly by NSF grant number PCM76-01490 and ERDA under contract with Union Carbide Corp.

**W-POS-F8** CHROMATIN ARCHITECTURE: SCANNING TRANSMISSION ELECTRON MICROSCOPY ( STEM ) STUDIES ON NUCLEOSOME FINE STRUCTURE. John P. Langmore* and John C. Wooley, Biophysics and Theoretical Biology Department, The University of Chicago, Chicago, Illinois, 60637.

Qualitative and quantitative results from our STEM dark field studies on unstained, unfixed chromatin have suggested a basic structure for nucleosomes, a 110A by 55A disc with an octameric histone core surrounded by 2 loops of DNA, each about 70 base pairs long. Specifically, center-to-center spacings of adjacent nucleosomes in chromatin aggregates indicate the width to be about 110A; the height of nucleosomes is about 55A based on the measured height of anhydrous nucleosomes (35A: L&W, PNAS,72, 2691, ]975) and the estimated internal hydration. Using the observed electron scattering properties we have determined the molecular weight of purified monomers and of nucleosomes found along H1-depleted chromatin, both of which are roughly 200,000 daltons; this result indicates (from the protein/DNA ratio) that the particles we visualize along chromatin fibers contain about 140 base pairs of DNA and about 110,000 daltons of histone. A detailed, quantitative analysis of the electron scattering from nucleosomes and from the internucleosomal (DNA) fiber indicates that there can not be any DNA at a radius of less than about 30A or greater than about 50A, the most probable location of the DNA (based on the peak in the scattering distribution) is roughly at a radius of 40A, and that there are 2 tightly-packed loops of DNA in a nucleosome. This conclusion is also supported by our observations of the molecular weight, height and DNA radius of nucleosomes, and by micrographs showing the DNA molecule entering and exiting from the same region of the nucleosome. (Supported by U.S. Atomic Energy Commission, Division of Biology and Medicine, by USPHS CA2739 and the Fay Hunter Cancer Research Fund)

**W-POS-F9** CHROMATIN ARCHITECTURE: NEUTRON AND X-RAY SCATTERING STUDIES ON NUCLEOSOME FINE STRUCTURE. John C. Wooley, John F. Pardon*, Brian M. Richards*, and David L. Worcester*, Searle Research Laboratories, High Wycombe, Bucks, U.K., and AERE Harwell, Oxfordshire, U.K. (Introduced by Su-yun Chung)

The shape of the nucleosome has been determined by analysis of neutron and x-ray wide-angle scattering. We have found only one model which is able to account for both x-ray and neutron scattering profiles. This model consists of a protein core of diameter 68A, height 40A, surrounded laterally by DNA to give a disc-like particle with overall width about 110A and height about 50A. The position of the DNA relative to the protein core was varied and best agreement between calculation and experiment obtained with DNA mainly confined to two annuli at the top and bottom of the nucleosome on the outside of a protein core, i.e., the DNA enters and leaves the monomer on one side and forms two loops around the histone octamer. One tetramer (of histones 2A, 2B, 3, and 4) or core protein (Wooley et al., J. Cell. Bio. 70, 294a, 1976) is presumably associated with one loop of DNA. Our observations on the disc-like structure of isolated core protein (which has a compact center, 50A by 16A, comprised of the C-terminal hydrophobic histone segments surrounded by the extended basic N-terminal tails; Richards et al., Current Chromosome Research, Jones and Berndham, eds., North Holland, 1976) support this model for the nucleosome; the model also accounts for previous x-ray and neutron diffraction patterns from chromatin and with our previous measurements of the DNA and protein radii of gyration in a nucleosome (Pardon et al., NAR 2, 2163, 1975), and is essentially that suggested by Langmore and Wooley (PNAS 72, 2691, 1975) based on observations by scanning transmission electron microscopy.

W-POS-F10 THE PHYSICAL ORGANIZATION OF THE COW GENOME AS DETERMINED BY ELECTRON MICROSCOPY, J. E. Mayfield and J. F. McKenna*, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213.

The size and distribution of A-T rich and short repetitive sequences were determined for cow DNA by visualizing either partially denatured or hybrid DNA with the electron microscope. DNA was partially denatured in 70% to 90% formamide in the presence of cytochrome c and spread according to standard techniques. Loops of partially denatured DNA were then scored according to size and distance between loops. At about 15% denaturation, where it is estimated that sequences with a G+C content of less than 35% will be denatured, the sequences are non-randomly arranged with about 50% of the denatured sequences occurring in about 20% of the total DNA. These sequences are much larger and less uniform in size than the middle repetitive sequences of cow suggesting that these two classes are unrelated. The middle repetitive DNA sequences were characterized by visualizing sheared and partially hybridized DNA of various sizes by both the aqueous and formamide basic protein film techniques. Intermediately repetitive DNA sequences in cow appear to belong to a single size class of about 275 nucleotide pairs in length with a possible variation of about $\pm$ 60 nucleotide pairs. Our analysis also indicates that these sequences comprise about 15% of the DNA and are separated by an average of 1200 nucleotide pairs of non-repetitive DNA.

W-POS-F11 INTERACTION OF BIS(1-ANILINO-8-NAPHTHALENESULFONATE) WITH E. COLI RNA POLYMERASE. F.Y.-H. Wu and C.-W. Wu, Dept. of Biophysics, Albert Einstein Coll. of Med. Bronx, NY 10461

Bis(ANS) is a dimer of 1-anilino-8-naphthalenesulfonate (ANS) with a C-C linkage at the 4,4' of naphthalene rings. More than 90% of RNA polymerase activity is inhibited by $10^{-5}$ M of bis(ANS) while no inhibition is observed with ANS at the same concentration. The concentration of bis(ANS) required for 50% inhibition is about 4 x $10^{-6}$M using various DNA templates. The inhibition can be partly prevented by preincubation of the enzyme with DNA and/or nucleoside triphosphates. At $10^{-5}$ M, bis(ANS) has no effect on the binding of RNA polymerase to DNA as measured by retention of the complex on nitrocellulose filters. However, little or no DNA retention is detected with $10^{-4}$ M bis(ANS). Kinetic studies and the differential effects of bis(ANS) on $^{3}$H-labeled-vs. $\gamma$-$^{32}$P-labeled nucleotide incorporations indicate that at $\leq 10^{-5}$ M bis(ANS) selectively inhibits RNA chain initiation compared to elongation. Bis-(ANS) is non-fluorescent in aqueous solution but fluoresces strongly upon binding to RNA polymerase. Also the tryptophan fluorescence of the enzyme is quenched by bis(ANS), implying that some aromatic amino acid residues maybe at or near the dye binding sites. The binding of bis(ANS) to RNA polymerase has been studied by a fluorimetric technique. The titration curve obtained shows multiple binding sites of the dye, which can be divided into two distinct classes: a class of 16$\sim$18 strong sites with $K_d$=2x$10^{-6}$ M and a class of 34$\sim$36 weak sites with $K_d$=2x$10^{-5}$ M. A glycerol-gradient centrifugation analysis at high salt conditions has revealed that RNA polymerase forms dimer and higher aggregates in the presence of $10^{-4}$ M bis(ANS) but remains as monomer (MW=500,000) at $10^{-5}$ M bis(ANS). Both the fluorescence and sedimentation results suggest that binding of bis(ANS) to the strong sites on RNA polymerase inhibits RNA chain initiation whereas binding to the weak sites induces aggregation of the enzyme which loses its ability to bind DNA template.

W-POS-F12 THE CONFORMATION OF E. COLI RIBOSOMAL PROTEINS. S.H. Allen* and K.-P. Wong+, Department of Biochemistry, University of Kansas Medical Center, Kansas City, KS 66103.

The conformation of ribosomal proteins free in solution and bound in the ribosomal particles has been studied by circular dichroism (CD) in the native medium and in the reconstitution condition. Analyses of the results obtained in reconstitution buffer indicate that 30S subunit ribosomal proteins free in the solution have approximately 29% $\alpha$-helix and 18% $\beta$-structure, whereas the proteins extracted from the 50S subunit have 23% $\alpha$-helix and 18% $\beta$-structure. The corresponding results obtained in native medium are: 22% $\alpha$-helix and 15% $\beta$-structure for the proteins from the 30S, and 13% $\alpha$-helix and 18% $\beta$-structure for the proteins from the 50S. Furthermore, the ribosomal proteins can be denatured by 6M guanidine hydrochloride and their CD spectra undergo changes in a manner similar to many of the known proteins which possess unique conformations. This observation suggests that at least many of the ribosomal proteins do have unique three-dimensional structures. The conformation of the proteins bound in the ribosomal particles in native medium and in reconstitution condition has been approximated from CD studies by subtracting the RNA contribution from the CD spectrum of the intact ribosomal particle. In reconstitution condition the ribosomal proteins in the 30S have 34% $\alpha$-helix and 7% $\beta$-structure, whereas the 50S proteins have 16% $\alpha$-helix and 6% $\beta$-structure. In the native medium the corresponding results are: 29% $\alpha$-helix and 5% $\beta$-structure for 30S proteins, and 13% $\alpha$-helix and 17% $\beta$-structure for the proteins in the 50S. The differences in the conformation may have significant relevance to the assembly of the proteins and the RNAs to form functional ribosomal particles. [Supported by NIH Grant GM 22962 and in part by HL 18905. +Career Development Awardee of the National Institute of General Medical Sciences (GM 70628)].