W-PM-H1 NMR FIELD-DEPENDENT STUDIES OF THE HYPERFINE SHIFTED HEME RESONANCES IN HEMOGLOBIN. M. E. Johnson and C. Ho, Department of Biophysics and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260.

For deoxygenated hemoglobin there are several hyperfine shifted proton resonances in the region 7 to 20 ppm downfield from HDO of which the two major ones at ∿ -18 and ∿ -12 ppm have been respectively assigned to heme methyls of the β and α chains. The line widths of these two resonances have been shown to be field-dependent, increasing from ∿ 170 Hz at 90 MHz to ∿ 280 Hz at 250 MHz to ∿ 400 Hz at 360 MHz. This dependence suggests that the line width is proportional to the square of the magnetic field with a zero field line width of ∿ 160 Hz. Upon oxygen ligation these resonances broaden substantially at 250 and 360 MHz, with the line broadening increasing as percentage ligation increases. The mechanism for this broadening is perhaps related to such effects as oxygen exchange kinetics or quaternary structural transition rates. Upon oxygen ligation in the presence of inositol hexaphosphate these resonances also exhibit an anomalous intensity decrease when compared to oxygen ligation in the absence of organic phosphate. The origin of these effects in terms of equilibrium and kinetic phenomena and their relation to the cooperativity of oxygenation in hemoglobin will be discussed. (Supported by NSF Grant (GB-37096X) and NIH Grants (HL-10383, RR-00292, F22HL-03005).

W-PM-H2 PROTON NMR STUDIES OF SICKLE CELL HEMOGLOBIN. K. A. Lin*, L. W.-M. Fung, and C. Ho, Department of Biophysics and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260.

High-resolution proton nuclear magnetic resonance (NMR) spectroscopy has been recognized as an excellent technique to investigate the structure-function relationships in human hemoglobins. It has provided detailed information about heme environments, the functional properties of individual hemes, and the subunit interactions during ligand binding. We have applied this technique to investigate sickle cell hemoglobin, Hb S (β6 Glu→Val). The hyperfine shifted resonances of the heme protons of ungelled Hb S and normal adult hemoglobin (Hb A) are identical, indicating that the heme environment of Hb S is similar to that of Hb A in the deoxy form. In a completely gelled sample, the heme proton resonances are too broad to be detected by the present experimental techniques. The ring-current shifted proton resonances of both HbCO S and HbO_2 S are identical to those of the corresponding liganded forms of Hb A, suggesting that the tertiary structures of the α and β heme pockets in liganded Hb S are very similar to those of liganded Hb A. The exchangeable proton resonances of the Hb samples in H_2O have provided valuable information about some of the subunit hydrogen bonds in the hemoglobin molecule. We have found no observable differences in the exchangeable proton resonances between Hb A and Hb S. On the other hand, we have observed significant differences in the aromatic proton resonances (especially the protons of histidine residues) between Hb A and Hb S suggesting that the amino acid substitution at β6 produces changes in the conformations of several surface residues of the Hb S molecule. The relationship between our NMR results and the altered functional properties of Hb S will be discussed. (Supported by NIH Grants HL-10383, HL-15165, and RR-00292).

W-PM-H3 NMR STUDIES OF ABNORMAL HUMAN HEMOGLOBINS. K. J. Wiechelman* and C. Ho, Department of Biophysics and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260.

High-resolution proton nuclear magnetic resonance (NMR) spectroscopy has been used to investigate the structural and functional properties of several abnormal human hemoglobins with mutations in the $\alpha_1\beta_2$ subunit interface. Mutations in the $\alpha_1\beta_2$ interface are of interest since the integrity of this region of the molecule is believed to be essential for normal cooperative oxygenation to occur. All known human hemoglobins with mutations in this region of the molecule have a decreased Hill coefficient and an altered oxygen affinity. Hence, the study of these hemoglobins may shed some light on the nature of the mechanism involved in the cooperative oxygenation of normal human hemoglobin. The hyperfine shifted proton resonances of hemoglobins Yakima [β99(G1) Asp→His], Kempsey [β99(G1) Asp→Asn], Chesapeake [α92(FG4) Arg→Leu], and Mälmo [β97(FG4) His→Gln] show that a mutation in the $\alpha_1\beta_2$ subunit interface can alter the heme environment of both the chain containing the mutation and the normal chain. The study of these mutants shows that hemoglobins in solution can assume both the oxy (R-type) and deoxy (T-type) quaternary structures and that organic phosphates can switch the R-form of some of these hemoglobins to the T-form. Inositol hexaphosphate can also induce differences in ligand affinity between the α and β hemes. Studies of the tertiary structure of amino acid residues in the vicinity of the heme show that the environment of the hemes in hemoglobins Kempsey, Yakima, and Mälmo differ from that of normal human hemoglobin. In contrast, the heme environment of Hb Chesapeake in both the oxy and carbonmonoxy forms is very similar to the corresponding forms of hemoglobin A. (Supported by NIH Grants HL-10383 and RR-00292).

W-PM-H4 INTERACTION OF CO_2 WITH ADULT HUMAN HEMOGLOBIN. J. S. Morrow*, R. J. Wittebort*, J. B. Matthew* and F. R. N. Gurd, Department of Chemistry, Indiana University, Bloomington, Indiana 47401

The interaction of CO_2 with adult human hemoglobin occurs primarily by carbamate formation with the NH_2-terminal valine residues of the α and β chains. Hemoglobin was equilibrated in bicarbonate buffers with $^{13}CO_2$ and observations were made by ^{13}C NMR spectrometry at 14.1kG and 23.5kG. Specific blocking of α and β chains by reaction with cyanate was used to identify the carbamino resonances. When the stripped, unmodified protein was equilibrated in the deoxy form, separate resonances at 29.2 ppm and 29.8 ppm upfield of external CS_2 were observed and attributed to the α and β chain NH_2-terminal sites, respectively. Values of the carbamino formation constant, Kc, and pKa for these groups were determined from the pH dependence of the integrated resonances. For the deoxy form pKc values of 4.6 to 4.7 for both chains were found in the ionic strength range 0.085 to 0.123 M. At pH 7.4, binding to the β chains is dominant because of the large difference in pKa between α and β chains. The sum of the carbamino derivatives of the separate chains agrees with accepted values obtained by classical methods. The interaction is reduced by conversion to the carboxyhemoglobin and cyanoferrihemoglobin forms, and the chemical shift distinction between the resonances is lost. Separate carbamino resonances near 28.4 ppm, attributable to ε-amino groups, make a small contribution up to pH 8 but become prominent at higher pH values. (Supported by PHS Grants HL-05556 and T1GM-1046).

W-PM-H5 COMPARISON OF THE LIGAND COMBINATION KINETICS FOR HIGH AFFINITY DERIVATIVES OF HUMAN HEMOGLOBIN. Alice De Young*, Anna L. Tan, Russell R. Pennelly*, and Robert W. Noble. Departments of Medicine and Biochemistry, SUNY at Buffalo, Veterans Administration Hospital, Buffalo, New York 14215.

The rate constant (ℓ_4') for carbon monoxide recombination with triliganded human hemoglobin ($HbA(CO)_3$) generated by partial flash photolysis has been measured as a function of pH. A definite pH dependence is observed with a value of 8.5×10^6 $M^{-1}sec^{-1}$ at pH 8 and 9, decreasing to 4.5×10^6 at pH 6, and increasing again to 6.5×10^6 at pH 5. The presence of either inorganic phosphate, citrate, borate or 2,3 diphosphoglycerate (DPG) has no significant effect on these rate constants or on the observed pH dependence. In contrast, addition of 1 mM inositol hexaphosphate, which is known to have a greater effect on HbA than does DPG, markedly decreases ℓ_4' particularly at pH 7 and below. The values of ℓ_4' were compared to the rates of CO recombination with two other high affinity derivatives, carboxypeptidase-A digested HbA (HbA-CPA) and isolated α chains of human hemoglobin. For both these derivatives the pH dependence of the CO recombination rate was much smaller than that for $HbA(CO)_3$, being constant from pH 9 to 7 and showing a slight (30%) increase upon going from pH 7 to 5. As expected, rates of CO recombination to α chains after full and partial photolysis are identical. This was not true for HbA-CPA for which the recombination rate after partial photolysis is approximately 30% faster than that observed after full photolysis. This suggests that ligand binding to HbA-CPA is cooperative and this has been confirmed by direct O_2 equilibrium measurements. Supported by USPHS Grant HL 12524 and funds from Veterans Administration Research Project #6098-01.

W-PM-H6 HEXAMETHYLENETETRAMINE: A POSITIVE HETEROTROPIC ALLOSTERIC EFFECTOR OF HUMAN HEMOGLOBIN. Anna L. Tan, Deborah E. Campbell*, and Robert W. Noble, Depts. of Medicine and Biochemistry, SUNY at Buffalo, Veterans Administration Hospital, Buffalo, NY.

We have found a compound, hexamethylenetetramine (HMT, empirical formula $C_6H_{12}N_4$) which, when used as a buffer component, raises the oxygen affinity of human hemoglobin previously stripped of organic phosphates. Oxygen equilibrium measurements were done using 25 mM cacodylate + 25 mM HMT from pH 5 to 6.5, 25 mM Bis-Tris + 25 mM HMT from pH 6 to 7.5 and 25 mM Tris + 25 mM HMT from pH 7.5 to 9.5 to test the HMT effect. These results were compared with those obtained using 50 mM cacodylate, Bis-Tris and Tris buffers as controls. The $P_{50}(O_2)$ for the HMT containing series was 2.5 mm Hg at pH 5, increased to 3.7 mm Hg at pH 5.6 then decreased to 0.29 mm Hg at pH 9. From pH 5 to 7.5, these oxygen affinities were 2 to 2.5-fold higher than the controls. Above pH 7.5, the HMT effect decreased and finally disappeared at pH 9. The value of the Hill parameter n clustered around 2.6 for both series throughout the pH range studied. This shows that the cooperativity of ligand binding was not affected by HMT. Addition of 7 mM 2,3 diphosphoglycerate to stripped Hb either in cacodylate or HMT buffers at pH 5.7 raised the $P_{50}(O_2)$ to 17.8 mm Hg. Once bound, the HMT effect was not reversed by passing through a Sephadex G-25 fine column equilibrated with 0.1 M NaCl and was partially reversed when the column was equilibrated with 10 mM Tris + 0.5 M NaCl at pH 9.

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W-PM-H7 THE INFLUENCE OF ALLOSTERIC EFFECTORS ON THE KINETICS OF THE FIRST STEP IN OXYGEN BINDING TO DEOXYHEMOGLOBIN. R. Pennathur, T. M. Schuster, Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268 and G. Ilgenfritz,* Institut fur Physikalische Chemie, Univ. Koln, Koln 41, Germany.

The rate of oxygen binding to human hemoglobin A has been measured at low O_2 saturation (<5%) using the temp-jump relaxation method in order to study the influence of phosphates on the properties of deoxyhemoglobin. The rate constants for this reaction k_f^4 and k_r^4 have been determined and are each increased in the presence of DPG or IHP, as compared with phosphate-free solvent conditions (0.1M HEPES; pH 7.0; 20 C). The value of k_r^4 is increased much more than k_f^4 but the magnitude of the increase of each is the same for saturating concentrations of either DPG or IHP. Although DPG and IHP bind to deoxyhemoglobin with very different affinities, these effectors produce the same changes in kinetics properties and therefore, the same change in K^4, the first binding constant, which is in contrast to the results at pH 7.4 of Imai (Biochem. 12 1493 (1973)). The structural changes produced by DPG and IHP are the same (Arnone, Nature 249 34 (1974)) and would predict the same changes in K^4. We conclude that this lowering of the O_2 affinity by phosphates results mainly from an increase in k_r^4 and that the deoxyhemoglobin structures with and without phosphates are functionally quite different. We expect this functional difference to exist in mutant deoxyhemoglobins in which phosphates induce a so-called R→T transition.

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W-PM-H8 STRUCTURAL KINETICS OF CO-BINDING IN HEMOGLOBIN†. F.A. Ferrone* and J.J. Hopfield, Department of Physics, Princeton University, Princeton, N.J. 08540

The transient circular dichroism[1] of hemoglobin as it rebinds CO following photodissociation has been followed in the L-band, 285-nm band, and Soret band. Events in the L-band, 285-nm band, and latter part of the Soret band agree with predictions made from static CD spectra and kinetic parameters of an MWC model. In the Soret band, an anomalous relaxation amplitude is observed in the first 10 msec, which can be consistently explained as arising from deoxy hemoglobin dimers, whose CD has not been previously observed. The significance of this difference between the deoxy dimer and tetramer will be discussed with respect to the structures reflected in the Soret CD.

†Work supported in part by NSF grant GH40474

[1]F.A. Ferrone, J.J. Hopfield and S.E. Schnatterly, Rev. Sci. Instrum. 45, 1392 (1974)

W-PM-H9 SURFACE ACTIVITY OF DIFFERENT HUMAN HEMOGLOBINS. Elbaum,D.* Harrington, J.*, Nagel, R.L.*,(intr. by T.T. Herskovits), Albert Einstein College of Medicine, Bronx, N.Y.

Studies by Asakura et al. (Nature, 244:437, 1973) and Roth et al. (Blood, 1975, in press) have shown that oxy Hb S and oxy Hb A exhibit very different rates of mechanical precipitation. Protein films were studied at water-air interface after injecting the protein solution into the subface and monitoring the surface pressure by Wilhelmy hanging plate technique. The surface activity (measured as surface pressure (π) vs time (t)) of Hb A, Hb S, Hb F and Hb C_{Harlem} were studied to understand the basis of their different rates of mechanical precipitation. The π-t isotherms indicate that the rate of mechanical precipitation parallels the rate of their alteration of surface pressure. In addition, ligand state has a definite effect: oxy Hb S is faster than oxy Hb A, but deoxy Hb S is quite similar to deoxy Hb A in their rates of surface pressure alteration. While quaternary conformation states (R and T) of Hb S have pronounced effects on the surface activity, changes within the R state of Hb S did not have any consequence for the kinetics of surface film formation as judged by lack of difference between Met-, CO- and oxy- ligand states of Hb S. In addition, isolated PMB-β^S chains are more surface active than PMB-β^A chains. While the temperature had relatively small effect on the surface activity of oxy Hb A, it had pronounced effect on the surface activity of Hb S. The effect of time of incubation on the rate of surface activity alteration was monitored as well. These findings suggest that surface tension kinetics and equilibrium differences between different mutants and conformers of human hemoglobin may be the basis for their differences in mechanical precipitability.

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W-PM-H10 THERMODYNAMIC STUDIES OF THE PRE-GELATION AGGREGATION OF SICKLE CELL HEMOGLOBIN. John Whitin‡ Pamela Hartzband* and Elizabeth R. Simons, Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02218.

In dilute solutions (< 50mg/ml), deoxygenated samples of HbS have been shown to undergo a pre-gelation aggregation. The rate of this aggregation, as determined from the intensity of light scattered perpendicularly to the entering light beam (612nm) by a thermostated and slowly stirred solution, has been obtained as a function of temperature. For comparison, the rate of aggregation of a deoxygenated solution of HbA, of identical concentration, has also been measured under exactly the same conditions. Oxygenated solutions, whether HbS or HbA, exhibit no aggregation under the same experimental conditions. This observation also proves that the aggregates cannot merely be denatured material as oxy Hb is less stable than deoxy Hb; in addition, redissolved aggregates of deoxy HbS and HbA exhibit normal spectra. From 23 to 37°, deoxy HbS exhibited an increase in rate of aggregation, with the latter giving rise to a linear Arrhenius plot and thus a positive energy of activation, + 44kcal/mol. Conversely, deoxy HbA exhibited a decrease in rate of aggregation with increasing temperature, leading again to linear Arrhenius plots but with a negative energy of activation, -9kcal/mol. These observations are fully consistent with an aggregation due to hydrophobic interactions by deoxy HbS, as postulated by Murayama, and are a consequence of the β^6 glu→val substitution. The aggregation of deoxy HbA was previously reported only for very high concentration; our data at low concentrations show that the mechanism is entirely different from that of deoxy HbS in that hydrophobic interactions are not involved while hydrogen bonds and ionic interactions may play an important role.

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W-PM-H11 SOLUBILITY STUDIES OF DEOXY SICKLE CELL HEMOGLOBIN. B. M. Fairchild*, T. Li*, W. N. Poillon*, and J. F. Bertles*, (Intr. by L. Katz), Department of Medicine, Columbia University, St. Luke's Hospital Center, New York, N. Y. 10025.

The solubility of deoxy-Hb S, at concentrations approaching that of hemoglobin in the erythrocyte, where polymer formation and regular paracrystalline arrays are observed, was measured as a function of temperature and pH. After temperature equilibration, the hemoglobin solutions were centrifuged at 270,000g, resulting in a well-demarcated pellet in equilibrium with the supernatant solution. The concentration of the supernatant was considered to be the solubility of deoxy-Hb S under the specific conditions of temperature and pH. The pH dependence of solubility at low temperature was almost zero, becoming more positive as the temperature was increased. The temperature dependence of solubility was negative below 22°C at pH 7.2, as well as over the range 6-38°C at pH 6.5. Strikingly, however, at the higher pH value, the temperature coefficient was zero above 22°C. The heat of solution, $\Delta \overline{H}$, which represents the enthalpy difference between deoxy-Hb S in solution and that of the crystalline and amorphous phases, shows a transition at 22°C over the entire pH range, indicative of a crystalline phase transition. Heat of solution, $\Delta \overline{H}$, varies from -3.5 kcal/mol to zero. Enthalpy differences of this magnitude indicate that the regular arrays of deoxy-Hb S polymers are stabilized by relatively weak forces.
(Supported by NIH grant HL 15293-03 and NIH contract N01-HB-2-2925.)

W-PM-H12 LIGHT-SCATTERING STUDIES OF DISSOCIATION OF HEMOGLOBIN FROM LUMBRICUS TERRESTRIS. D. J. Goss, L. J. Parkhurst, and H. Goerisch, Dept. of Chemistry, University of Nebraska, Lincoln, Nebraska 68508.

The kinetics of the pH dependent dissociation of the 3×10^6 mol. wt. hemoglobin (the duodecamer) from Lumbricus terrestris (the earthworm) have been studied in a specially constructed light-scattering stopped-flow directly interfaced to a mini-computer. The ligand-dependent rates of dissociation for the CO and O_2-bound forms are consistent with the mechanism: $Hb_{12} \rightarrow 2\ Hb_6 \rightarrow 12\ Hb$. At a hemoglobin concentration 120 μM in heme, pH 7, met-hemoglobin appears to be hexameric. When the pH is rapidly changed from 7 to 10.3, met-hemoglobin dissociation kinetics are consistent with the mechanism: $2Hb_6 \rightarrow 12\ Hb$. The experimental dissociation curves were fit satisfactorily with the sequential model using a Fletcher-Powell sum of squares minimization program on a Super Nova computer with 20K of memory. The activation energy for the dissociation process was found to be about 30 kcal/mole for the CO and O_2-bound forms of the hemoglobin and about 26 kcal/mole for met-hemoglobin. When CO replaces O_2 as the ligand, the dissociation rate increases by a factor of four. When the hemoglobin is oxidized to the met form the dissociation is twenty times faster than the initial oxy hemoglobin dissociation rate. The rate constants k_1, k_2 (sec^{-1}) at 21° were, oxy: 0.26, 0.01; CO: 0.70, 0.04; met: (-), 5.2. (This work was supported by: Grant No. HL 15284-03 from the NIH, NSF Grant No. 2054 to Dept. of Chemistry, a grant from the Research Corporation, and a Grant-in-Aid from the Nebraska Heart Association.)

W-PM-H13 KINETICS OF THE REACTION OF PARA-HYDROXYMERCURIBENZOATE WITH β-CHAINS OF HUMAN HEMOGLOBIN. H. Goerisch, G. Geraci* and L.J. Parkhurst, Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68508; and Laboratory of Molecular Embryology, Consiglio Nazionale delle Ricerche, 80072 Arco Felice, Napoli, Italy.

Isolated β-chains of human hemoglobin are known to be tetrameric. After reaction with PMB the β-chains are monomeric. The reaction kinetics were studied in a specially designed light-scattering stopped-flow apparatus also equipped with absorption optics for monitoring the absorbance change at 254 nm. This device is directly interfaced to a mini-computer which permits on-line data acquisition, reduction and analysis. With respect to the rate of PMB binding, there exist two classes of SH groups. The first class binds PMB very rapidly with a second order rate constant of ca. 1×10^7 M^{-1} sec^{-1}, while the second class of SH groups reacts after a lag phase and with a much slower rate. The pseudo-first-order rate constant of the latter process is not linear with PMB concentration. Preliminary stopped-flow light-scattering experiments show that the dissociation of the tetramer occurs with a first-order rate constant of ca. 2.5 sec^{-1}. The Fletcher-Powell non-linear minimization algorithm for parameter variation was used to explore the suitability of various sequential kinetic mechanisms for description of and precise fit to the absorption data. (This work was supported by: Grant No. HL 15284-03 from the NIH, NSF Grant No. 2054 to the Department of Chemistry, a grant from the Research Corporation, a Grant-in-Aid from the Nebraska Heart Association, grants from the Research Council of the Univ. of Neb., the NSF-CNR US-Italy Joint Scientific Program, and a NATO-fellowship to H.G.)

W-PM-H14 THERMODYNAMIC ANALYSIS OF THE ACCURACY OF OXYGEN BINDING CURVES OF HEMOGLOBINS. Attila Szabo, Department of Chemistry, Indiana University Bloomington, Indiana 47401, and Martin Karplus*, Laboratoire de Chimie Theorique, Universite de Paris VII, Paris, France.

Accurate oxygen binding curves of normal and modified hemoglobins have recently been obtained by Imai and coworkers. Analysis of the changes of these curves produced by altering external conditions such as pH and organic phosphate concentration can lead to insights into the nature of cooperativity and serve as a basis to differentiate various stereochemical models for the mechanism of action of hemoglobin. The validity of such studies is crucially dependent on the accuracy of the experimentally determined data. For the case of binding curves of hemoglobin measured in the presence of different concentrations of organic phosphates we show, using a model-independent thermodynamic argument, that the quoted error bars on the Adair constants must be too small. Hence, conclusions based on these Adair constants must be regarded with caution. In addition, we show that the asymmetry of the Hill plots of the mixed state valency hybrids unambiguously shows that the systems are heterogeneous. Consequently these experiments on the functional properties of valency hybrids are not suited to make quantatative arguments for or against a particular model of cooperativity in hemoglobin.

W-PM-I1 RESONANCE RAMAN SCATTERING FROM ACTINOMYCIN-D AND ITS NUCLEOSIDE COMPLEXES. Bruce P. Gaber and William S. Craig*, Department of Natural Sciences, The University of Michigan-Dearborn, Dearborn, Michigan 48128.

The chromopeptide antibiotic actinomycin-D forms a specific intercalation complex with DNA, resulting in the blocking of protein synthesis at the level of transcription. The drug is a potent antitumor agent, but has achieved only limited clinical application due to its extreme toxicity. An analysis of the vibrational spectra of the drug and its nucleoside complexes could provide information valuable in describing the nature of drug-target interactions. Laser excitation (4579A) within the visible electronic transition (λmax=440 nm) of actinomycin-D's phenoxazone chromophore, reveals four intense resonance raman bands. The bands, at 1264cm^{-1}, 1402cm^{-1}, 1488cm^{-1} and a shoulder at 1500cm^{-1} arise from ring vibrational modes of the chromophore. The modes prove to be responsive, to some extent, to the interactions involved in intercalation with purines. For example, upon complex formation with deoxyguanosine, the intense peak at 1488cm^{-1} narrows and shows a marked increase in intensity while the shoulder at 1500cm^{-1} is significantly diminished. (Supported in part by grants from Research Corporation and U. of Mich., Div. Res. Dev. Adm., Grant #360706)

W-PM-I2 FLUORESCENCE OF THE Y BASE IN YEAST tRNA^{Phe} ORTHORHOMBIC CRYSTALS. Richard Langlois*, Sung-Hou Kim, Charles R. Cantor, Departments of Chemistry and Biological Sciences, Columbia University, New York, N.Y. 10027, and Department of Biochemistry, Duke University, Durham, N.C., 27710.

The fluorescent properties of the Y base of yeast tRNA^{Phe} are known to be quite sensitive to the environment. The fluorescence lifetime of yeast tRNA^{Phe} is identical in orthorhombic crystals and in the mother liquor from which these crystals are grown. It is 10% higher than the lifetime observed in dilute solution of tRNA. This small change is a solvent effect due to isopropanol in the crystallization medium since isopropanol does not change the accessibility of the chromophore of the Y base as measured by iodide quenching rates in solution. Thus, within the limits of the sensitivity of the method, the Y chromophore occupies the same environment in solution and in the crystal. The rate of iodide quenching in tRNA^{Phe} is much less than in oligonucleotide fragments containing the Y base suggesting that the Y chromophore is less exposed in the intact tRNA. This is consistent with the base stacking observed in the anticodon of yeast tRNA^{Phe} by x-ray diffraction

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W-PM-I3 STRUCTURAL RELATIONSHIPS IN TRANSFER RNA'S STUDIED BY ISOTOPE LABELING. H.J.P. Schoemaker*, R.C. Gamble*, and P.R. Schimmel, Biology & Chemistry, M.I.T., Cambridge, Ma. 02139.

The local environments at the purine sites in several specific tRNAs have been probed by an extension of the tritium labeling approach described by R.C. Gamble and P.R. Schimmel (1974) Proc. Nat'l. Acad. Sci. U.S. 71, 1356-1360. The basic rationale is to monitor the rate of tritium incorporation into purine C-8 positions distributed throughout the molecule. The earlier work showed that the labeling rate is sensitive to conformation and structure. The nucleic acid is incubated in ^3H_2O and then isolated and digested with T1 ribonuclease. The T1 fragments are separated and each is subsequently digested to mononucleotides with T2 ribonuclease. Specific activities of separated A and G residues from all of the T1 fragments are then determined. (Digestions and separations are done under conditions where exchange out does not occur.) Although the purines in thermally denatured tRNAs, and in oligonucleotide fragments at 37°, incorporate tritium at a rate comparable to free purine mononucleotides, labeling of purines in the folded tRNAs examined is markedly dependent on the positions of the purines in the structure. At 37° in the presence of Mg^{2+} purines in cloverleaf helical sections are markedly retarded in their rate of tritium incorporation, even more so than those in double stranded DNA and synthetic poly A:poly U. While there is a relatively small retardation of tritium incorporation in some purines in cloverleaf single-stranded sections, others show a marked reduction. These happen to correspond to some of the bases involved in tertiary interactions in the crystal structure. (Supported by USPHS. NIH Grant GM-15539.)

W-PM-I4 SECONDARY STRUCTURE OF PURIFIED OVALBUMIN mRNA. Nguyen T. Van, James W. Holder*, Anthony R. Means*, Savio L.C. Woo*, and Bert W. O'Malley* Department of Cell Biology, Baylor College of Medicine, Houston, Texas77025

Two highly purified and biologically active eucaryotic mRNAs, the Ovalbumin mRNA and the Globin mRNA, were studied by three different techniques: thermal denaturation, circular dichroism and ethidium bromide probing.

1) The Ov mRNA, at low salt is half denatured at room temperature whereas under the same condition, the Hb mRNA and the viral MS2 RNA are substantially undenatured. For the Ov mRNA, the melting temperature is a linear function of pM over the range of 0.001M-1.5M, KCl, with a slope of -18C/pM unit, quite similar to the slope obtained for chick liver DNA but 40% greater than the slope for Hb mRNA. The plot of Tm vs % G+C yields a straight line for Hb mRNA, 4s tRNA, 18s and 28s rRNAs but the Tm of homocopolymers are not coincident with this straight line. Using this plot to estimate the G-C content will thus give an overestimate for Ov mRNA and an underestimate for MS2 RNA.

2) The circular dichroism data indicated that the positive ellipticity at 267nm is significantly lower for Ov mRNA than for the Hb mRNA.

3) The ethidium bromide probing showed that at least 25% of the sequences of the two mRNAs exist as double helices when analysed by the Scatchard technique.

In conclusion, the Ov mRNA was shown to be more thermolabile and contain less base stacking than Hb mRNA. Both mRNAs have a similar amount of double helical structure (∿25%). The coexistence of helical structure and unstacked sequences might be of consequence in the biological function of Ov mRNA.

This work was supported by NIH grants HD 7495, HD 8188, HD 7857 and by the Ford Foundation grants.

W-PM-I5 CONFORMATIONAL CORRELATION BETWEEN THE SUGAR PUCKER AND THE INTERNUCLEOTIDE P-O BONDS IN POLYNUCLEOTIDES. N. Yathindra* and M. Sundaralingam, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706.

We have shown earlier from semiempirical conformational energy calculations that the phosphodiester is most restricted in a dinucleoside triphosphate (pXpXp) compared to a dinucleoside mono- (XpX) or diphosphate (pXpX or XpXp) indicating that interactions between the phosphate groups one removed from each other in a polynucleotide play an important role in limiting the orientations of the nucleotide residues about the P-O bonds. More recently we have extended these studies to all possible conformational combinations of the sugar pucker in a dinucleoside triphosphate. These studies have provided information on the dependence of the internucleotide P-O bond conformations on the mode of sugar pucker. It is found that the g^-g^- phosphodiester conformation which leads to the stacked, helical structure turns out to be one of the more important backbone conformations. Thus, the helical conformation is expected to be a predominant conformer in nucleic acid secondary and tertiary structures. The effect of the sugar pucker on the conformational priorities of the other phosphodiester conformations (tg^-, g^-t, tg^+, g^+g^-) yield important insights into the molecular mechanics of polynucleotide chain folding. The effect of the distorted and less favored nucleotide conformations on the internucleotide P-O bond rotations is also discussed.

Research supported by grants GM-17378 and GM-18455.

W-PM-I6 THEORETICAL STUDIES ON THE CONFORMATION OF CYTIDINE 2'3' CYCLIC PHOSPHATE : AN INTERMEDIATE IN RNase MEDIATED HYDROLYSIS. V. Renugopalakrishnan, Dept. of Chemistry, Rutgers, The State University of New Jersey, Newark, N.J. 07102.

A theoretical study of the conformation of cytidine 2'3' cyclic phosphate - an intermediate implicated in the hydrolysis of RNA by RNase, is presented in this paper. The conformational energy calculations were performed on the cyclic nucleotide using classical potential energy function, assuming the total energy to be additive. The monopole charges used in the calculation of electrostatic energy were derived from a CNDO/2 study of the cyclic nucleotide. CNDO/2 method predicts a dipole moment of 4.29 debyes for cytidine 2'3' cyclic phosphate which is roughly in the range obtained for the dipole moments of nucleosides by experimental methods. Total energy of the cyclic nucleotide as a function of the torsional angle χ was determined. Theoretical calculations unambigously show the minimum energy conformation corresponding to $\chi = -105°$, occuring in the syn region. This prediction is in good agreement with x-ray studies of Coulter[1]. A second minimum is predicted to occur in the anti region. The syn conformer is stabler than the anti conformer by 0.5 kcals/mole. It is observed that the electrostatic energy considerably stabilises the structure. The structure of cyclic nucleotide in relationship to the mechanism of RNase action is discussed.

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W-PM-I7 THE PHOSPHODIESTER CONFORMATION IN RANDOMLY COILING POLYNUCLEOTIDES. W.K. Olson, Department of Chemistry, Douglass College, Rutgers, the State University, New Brunswick, New Jersey 08903.

To date there has been no direct experimental measure of the conformation of the phosphodiester rotations in a randomly coiling polynucleotide. Theoretical estimates of conformational energy predict the P-O rotations (ω' and ω) to be the principal source of flexibility in the polynucleotide chain, but the various methods disagree with each other and also with the available evidence from crystallographic studies in their prediction of the preferred values of these two angles. Indirect measurement of the phosphodiester conformation, however, may be attained by comparison of mean square dimensions of polynucleotides calculated on the basis of the various theoretical models and the solid state structural data with experimentally measured dimensions. Results of such an analysis indicate that the random coil conformation is very different from the familiar stacked structure of polynucleotide helices. The high degree of extension observed in randomly coiling polynucleotides requires a large proportion of trans linkages about the P-O bonds of the chain.

W-PM-I8 PROTON MAGNETIC RESONANCE STUDIES OF URIDYLYL-(3'-5')-ADENOSINE. CONFORMATIONAL ANALYSIS. F. S. Ezra and S. S. Danyluk, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, N. S. Kondo, Department of Chemistry, Federal City College, Washington, D.C. 20005

The unambiguous proton magnetic resonance (pmr) spectral assignments of uridylyl-(3'-5')-adenosine (UpA) by specifically deuterating one of the residues in the dimer was recently reported. We have currently undertaken a detailed study of UpA in solution by Fourier transform pmr at 220 MHz. Both deuterated analogues, 5,6,1',2',3',4',5',5'-octadeuteriouridylyl-(3'-5')-adenosine (*UpA) and uridylyl-(3'-5')-2,1',2',3',4',5',5'-heptadeuterioadenosine (UpA*), have been synthesized and utilized in the measurement of the various nmr parameters. Key structural features such as equilibrium conformations of the ribose ring, exocyclic group rotamer populations and relative base ring orientations have been deduced from chemical shifts and spin spin coupling constants. The results are compatible with those of the UpA single crystal structure from X-ray diffraction data. Temperature and pH dependence studies suggest that conformational changes occur in the sugar ring and ribophosphate backbone.

Acknowledgement: This research was supported by the U.S. Atomic Energy Commission.

W-PM-I9 THE INFLUENCE OF BASE SEQUENCE ON THE CONFORMATIONS OF RNA SUBUNITS: POTENTIAL ENERGY CALCULATIONS FOR ApA, CpC, GpG, and UpU. S. Broyde and R. M. Wartell, Georgia Institute of Technology, and S. D. Stellman and B. Hingerty,*Princeton University. Potential energy calculations have been made for the ribodinucleoside monophosphates ApA, CpC, GpG and UpU. Contributions to the energy from Van der Waals, electrostatic and torsional potentials were included. The energy was minimized, with all eight conformational angles as simultaneously variable parameters. At the global minimum, ApA and CpC have conformations like double helical RNA: the angles ω' and ω, about the oxygen-phosphorus bonds, are g-g-, the sugar pucker is C3'-endo, and the bases are anti. GpG and UpU have the ω', ω angle pair near the g-t region at the global minimum, and for GpG the bases are syn. Energy contour maps for $\omega'$and ω show two broad, low energy regions for ApA, CpC and UpU: one is g-g-, and the second encompasses g+g+ and the region near g-t within a single low energy contour. The latter conformation could generate a turn if incorporated in helical RNA. The two low energy regions are connected by a path at 10-13 kcal./mole. For GpG, with bases syn, however, only a small low energy region near g-t is found. The helical RNA conformation is 8.5 kcal./mole higher for this molecule. Thus, the base sequence is shown to influence the conformations adopted by dinucleoside phosphates.

W-PM-I10 PROTON MAGNETIC RESONANCE STUDIES ON ApApA. THE UNAMBIGUOUS ASSIGNMENT OF BASE AND ANOMERIC PROTON SIGNALS. N. S. Kondo, Department of Chemistry, Federal City College, Washington, D. C. 20005 and S. S. Danyluk, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

One of the major problems encountered in studying oligonucleotides by proton magnetic resonance spectroscopy is that of making unambiguous signal assignments. We are currently dealing with this problem by preparing specifically deuterated oligonucleotides. Spectral comparison between partially deuterated and fully protonated compounds results in direct peak assignments for the latter.

Utilizing this approach we have been able to assign the complete pmr spectra of a variety of dinucleoside monophosphates. This work has now been extended to trinucleoside diphosphates and the present communication reports an assignment of all base and anomeric peaks for ApApA.

The assignment was facilitated by synthesis of deuterated analogs *ApApA, Ap*ApA and trimers with H-8 replaced by D-8 at selected nucleotides An assessment of chemical shifts and anomeric proton coupling constants derived from the assignment has given insight into intramolecular base stacking interaction and ribose ring conformation in ApApA.

Acknowledgment: This work was supported by the U. S. Atomic Enery Commission.

*indicate residue fully deuterated.

W-PM-I11 ELECTRON MICROSCOPY OF RIBOSOMAL RNA MOUNTED IN THE ABSENCE OF A PROTEIN FILM: IMPLICATIONS FOR SECONDARY STRUCTURE.
T.R. Johnson* and J.D. Caston, Department of Anatomy and Developmental Biology Center, Case Western Reserve University, Cleveland, Ohio 44106

Ribosomal RNA isolated from chick embryos by phenol extraction and further purified by sucrose gradient centrifugation was adsorbed onto electron microscope grids pretreated with the quaternary ammonium salt benzalkonium chloride. Staining with uranyl acetate in organic solvents yielded adequate contrast for microscopy. Molecules adsorbed from moderate salt concentrations appeared visually as bush-like structures which showed evidence of intramolecular interactions although no readily apparent regularities were present. Both RNA species, when adsorbed from very low salt concentrations or from high concentrations of urea, assumed reproducible shapes which allowed measurements to be made. The 28S species appeared as a linear axis with projections extending out from it at definite positions, dividing the molecule into 4 characteristic regions, while the 18S species under the same conditions showed no projections. Acrylamide gel electrophoresis showed the RNA to be essentially free of single-strand breaks. The lengths of both species agree well with predictions based on a hairpin loop model for ribosomal RNA in solution, derived from spectrophotometric studies by other workers. The lengths, however, are much shorter than those observed with other electron microscope techniques.

W-PM-I12 DETECTION OF THE NITROGEN MUSTARD ALKYLATION OF PURINES IN AQUEOUS SOLUTION BY RAMAN SPECTROSCOPY.
Samir Mansy* and Warner L. Peticolas, Department of Chemistry, University of Oregon, Eugene, Oregon 97403.

Alkylation of the purines, inosine, guanosine and adenosine in nucleosides, nucleotides and polynucleotides has been followed in aqueous solution by means of Raman spectroscopy. Since two moles of HCl are liberated for each mole of bifunctional alkylating agent reacted, it is necessary to carry out their reactions in a pH-stat at constant pH. The position of alkylation on the purine ring is a function of the pH. Identification of the species is obtained by comparison of the Raman spectrum of the product with the Raman spectrum of the corresponding methylated purine derivative. Stoicheometry of the reaction is obtained by varying the molar ratios of the reactants. Thus at neutral pH or higher the mustard alkylates the N(1) of inosine and guanosine with a displacement of the N(1) proton giving a Raman spectrum identical to that of the corresponding N(1) methyl purine. At low pH, the alkylation occurs at the N(7) position. Thus Raman spectroscopy provides a method for following the alkylation of purines in aqueous solution by an important class compounds known to have a lethal effect on melanoma cancer cells.

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W-PM-113 TRANSFER RNA IN THE ELECTRON MICROSCOPE. A.P. Korn* and F.P. Ottensmeyer (Intr. by J.E. Till), Department of Medical Biophysics, University of Toronto, Toronto, Canada M4X 1K9.

Dark field transmission electron microscopy can reveal the fine structure of macromolecules without the prior treatment of the specimen with staining or shadowing materials. With this technique, it is the molecule itself, rather than the large heavy-metal particles surrounding or pervading it that is visualized. The possibility arises, therefore, of determining structural and conformational information of biological macromolecules in a relatively simple and routine way. Towards this goal, transfer RNA has been studied as a test specimen for the technique. The complete structure of yeast phenylalamine tRNA in the crystalline state is known from X-ray diffraction. Correlation of the results of the two techniques - electron microscopy and X-ray diffraction - should be attempted before models for other tRNA's based on electron microscopic images are suggested. Several images from an unfractionated sample of yeast tRNA show that such a correlation may be possible. For the purpose of the structure determination of other tRNA species, unique site heavy atom markers are being used to identify specific regions of the molecule. A three heavy atom complex composed of one molecule of osmium tetroxide and two molecules of 3-pyridylmercuric acetate labels unfractionated tRNA at an average of 2.8±.6 sites, assayed chemically. An electron microscopic study has verified this figure. Another marker composed of osmium tetroxide and two molecules of 3,5-bis (mercuric acdetate)α-pyridone provides a five heavy atom marker. In addition mercuric acetate, a single heavy atom marker, has been shown to react with nucleic acids with a specificity for cytosine and uracil.

W-PM-J1 EQUILIBRIUM AND KINETIC STUDIES OF DNA MELTING: LOW MOLECULAR WEIGHT DNA. Charles P. Woodbury and M. Thomas Record, Jr., Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706.

At acid pH, the GC dependence of the denaturation temperature (T_m) of DNA is diminished. The effect increases in importance with decreasing pH. Presumably the GC pair is preferentially destabilized by acid, since both of its members are susceptable to protonation, whereas T is not. The pH effect and the effect of tetraalkyl ammonium salts (TEA, TMA) (Melchior and von Hippel (1973), Proc. National Acad. Sci., 70, 298-302) are cumulative, and, by proper adjustment of the pH and TEA concentration, GC independent denaturation conditions can be achieved for any choice of temperature. We have investigated the denaturation transition of calf thymus DNA and its pauci disperse, sonic fragments (molecular weight 45,000 - 300,000 daltons) under these conditions. Under GC-independent conditions, the T_m decreases and the transition breadth increases with decreasing molecular weight. The kinetics of denaturation of these fractionated fragments are under current investigation. (Supported by NSF Grant GB-43249.)

W-PM-J2 EQUILIBRIUM AND KINETIC STUDIES OF DNA MELTING: HIGH MOLECULAR WEIGHT DNA. Harlee S. Strauss* and M. Thomas Record, Jr., Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706.

The kinetics of the helix coil transition of T4 and nonglucosylated T4 DNA have been investigated in alkaline and neutral tetraalkyl ammonium salt solutions, respectively, under conditions where the GC dependence of the denaturation temperature is reduced or eliminated. The kinetics are complex, showing a spectrum of decay times in the approach to denaturation equilibrium. The rate constant for the slowest decay process decreases with increasing extent of denaturation, as observed previously by Record and Zimm (Biopolymers 11, 1435-84 (1972)) in a formamide-water solvent where the melting temperature is base-composition dependent. Most aspects of the kinetic behavior, including the qualitative features of the decay curve, the functional interrelationships of the rate constants, and the magnitudes of the rate constants, are similar in the base-composition-dependent and -independent solvents. We conclude that base compositional effects are unimportant in the rate of denaturation of T4 DNA, and that hydrodynamically limited unwinding determines the kinetics of the denaturation reaction. (Supported by NSF Grant GB-43249.)

W-PM-J3 DNA REASSOCIATION KINETICS AND CHROMOSOME STRUCTURE IN THE CRAB, LIBINIA EMARGINATA, J. C. Vaughn, Department of Zoology, Miami University, Oxford, Ohio 45056.

DNA reassociation kinetics have been partially elucidated for the crab L. emarginata (testes) and for the bovine genome (thymus), the latter being our standard. The crab genome contains three readily resolvable kinetic components. The first comprises 25% of the genome, is very rapidly reassociating, and is represented by more than 4×10^5 nucleotide sequences averaging 250 nucleotides each per haploid genome. Thermal denaturation studies show that 33% of this component is dAT satellite. The second kinetic component comprises 45% of the genome, reassociates at an intermediate rate, and is on the average represented by 4×10^3 copies. The slowest reassociating component (30% of the crab genome), after correcting for repeated sequences and % (G+C), has a cot 1/2 of 2.24×10^3, whereas the corrected cot 1/2 for the corresponding bovine component is 3.12×10^3. The quantitative cytochemical observation that the bovine haploid genome, whose chromatids are thought to be uninemic [Laird, Chromosoma 32, 378 (1971)], is almost precisely 1.39 times as large as the crab haploid genome leads to the conclusion that there is insufficient DNA in the haploid crab genome to support a binemic or polynemic chromatid structure, and that therefore the crab chromatids are also themselves uninemic structures. Accordingly, the slowest reassociating crab DNA sequences are represented only once each per haploid genome. (Supported by a Faculty Research Grant from Miami University).

W-PM-J4 PREFERENTIAL INTERACTIONS OF NATIVE AND DENATURED CALF THYMUS DNA IN AQUEOUS Na_2SO_4 SOLUTIONS OF VARYING IONIC STRENGTH. J. C. C. Fu* and D. W. Gruenwedel, Department of Food Science and Technology, University of California, Davis, California 95616.

Precision density measurements were performed on NaDNA-Na_2SO_4 mixtures, and the so-called density increment $(\partial\rho/\partial c_2)^\circ_\mu$ determined as a function of Na_2SO_4 molality. The partial specific volumes ($\bar{v}_2$) of native, heat-denatured, and alkali-denatured DNA were also determined with the help of density measurements. All $\bar{v}_2$ values increase with increasing Na_2SO_4 concentration, irrespective of the secondary structure of DNA. $\bar{v}_2$ of both native and heat-denatured DNA extrapolates to a value of 0.50_0 ml/g at vanishing salt concentration, neutral pH, and 25°C. $\bar{v}_2$ of alkali-denatured DNA (pH 12.6, 25°C) extrapolates to a smaller value.

Distribution coefficients of diffusible components, expressed in terms of preferential solvation and preferential salt interaction, were evaluated from the density increments, solvent densities, and partial specific volumes of all solution components. All interaction parameters depend strongly on salt concentration and on the conformation of DNA. Some of the data can be explained on the basis of "site-specific" ion binding. Supported by USPHS, NIH, GM 16282.

W-PM-J5 THEORETICAL CALCULATIONS OF THE MELTING TRANSITION OF $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ AND RELATED OLIGOMERS. R.M. Wartell, School of Physics, Georgia Inst. of Tech. , Atlanta,Georgia 30332

Theoretical calculations have been carried out to interpret the melting transitions of the block duplex polymer $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$. Experimental studies have been previously reported on this polymer (Burd and Wells,Fed. Proc. Abst. 53 , 1423,1974). This synthetic DNA provides a model system for examining the transmission of conformational effects along contiguous DNA sections. The studies showed broad monophasic melting transitions in 0.02M and 0.1M sodium ion concentration. In 1.0M sodium ion there was an indication of a two phase melting process. The midpoints of the transitions in 0.02M and 0.1M sodium ion were 51 C and 62 C respectively. Theoretical predictions of the melting curves of this DNA have been made with the nearest neighbor Ising model. This calculation included nearest neighbor interactions between base pairs and the effect of strand concentration on duplex dissociation. Employing thermodynamic parameters evaluated from the helix coil transitions of poly(dA)•poly(dT) and poly(dG)•poly(dC) and a strand dissociation constant of about .001, the theory predicts T_m's of 52 C and 63 C for $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ in 0.02M and 0.1M sodium ion. The predicted transitions are monophasic and in good agreement with the experimental curves. Varying the dissociation constant from .0001 to.1 does not effect the monophasic character of the transition. Results will be presented on the influence of the lengths of the AT and GC blocks on the melting transition.

W-PM-J6 THERMAL SUBPOPULATIONS IN NATURAL DNAS. D.L. Vizard and A.T. Ansevin, Department of Physics, M.D. Anderson Hospital & Tumor Institute of The University of Texas System Cancer Center, Houston, Texas 77025.

The automated, high resolution analysis of DNA thermal denaturation is capable of detecting small thermal subpopulations in virtually all natural DNA samples tested. For convenience, we shall refer to these subpopulations as thermalites. The appearance of observed thermalites is determined by the nature of the DNA source: 1) Bacterophage DNAs of unique sequence display thermalites representative of subsequences of various stabilites, some of which have thermal transitions as narrow as synthetic homopolymers. 2) Bacterial DNAs, which are collections of fragments from a very large unique sequence, generally display an asymmetric or nonrandom distribution about a single modal temperature; exceptional cases display discrete thermalites. Our current limit of resolution precludes distinguishing most subsequences of bacterial DNAs. 3) Eukaryotic DNAs, which are collections of fragments from a relatively heterogeneous mix of unique, moderately repeated and highly repeated sequences, display a mixture of broad and narrow thermalites constituting a range of mass fractions extending down to minute quantities detectable only by subfractionation of the genome. The appearance of thermalites is a strong function of DNA molecular weight, ionic strength, and the nature of the supporting cation. The interdependence of these parameters is complex, but may yield additional information about the organization of the DNA sequences which the thermalites reflect. (Supported by the Robert A. Welch Foundation, G-290).

W-PM-J7 THE NATURE OF THE METHYL GREEN-DNA COMPLEX. Anne K. Krey and Fred E. Hahn,* Department of Molecular Biology, Walter Reed Army Institute of Research, Washington, D.C. 20012

The triphenylmethane dye methyl green, MG, forms a stable complex with double-stranded DNA while, in contrast, free MG in non-acidic solution undergoes a molecular conversion to its carbinol base with progressive loss of color. Native calf thymus DNA shifted MG's absorption maximum to longer wavelengths and produced an increased absorption intensity which was independent of time, perhaps indicating placement of methyl green into the hydrophobic environment in DNA and thereby preventing its intramolecular conversion. DNA rendered optically active all of MG's electronic transitions. For 3 of these transitions, bound MG exhibited a flow dichroism of same sign and magnitude as that of DNA, while the effect for the dye's longest wavelength absorption was smaller and of opposite sign. We conclude for the arrangement of methyl green in DNA a distinct orientation, perhaps through intercalation of 2 of the dye's phenyl ring systems. Poly d(A-T) produced the same hyperchromic effect as DNA and a larger bathochromic shift for MG while in the presence of single-stranded ϕX174 DNA and t-RNA the absorbancy of the dye decreased. These observations are in accord with the known preference of methyl green for the double-helical structure of DNA. In contrast, poly dG·dC failed to prevent decay of MG; the conformation of duplex poly dG·dC differs from that of the duplex of poly d(A-T) and of DNA and we infer a unique double-helical conformation for the stabilization of the dye. Methyl green was displaced from DNA by DNA-complexing substances, foremost by those which cause an extension of the double helix through intercalation. A conformational change, produced by alcohol, from the B to the A form of DNA, also released MG. We conclude a preferential binding of methyl green to the B conformation of native DNA.

W-PM-J8 PRESSURE-TEMPERATURE STABILITY OF DNA IN NEUTRAL SALT SOLUTIONS, R. M. Macleod, Department of Biochemistry, University of Tennessee Center for Health Sciences, Memphis, Tennessee 38163 and S. A. Hawley*, Jefferson Physical Laboratory, Harvard University, Cambridge, Massachusetts 02138.

The effect of pressure on the thermally induced melting behavior of native DNA has been examined as a function of base composition and neutral salt concentration. The results indicate that the transition volume vanishes over a relatively narrow range of temperature and salt concentration and provide a relatively simple explanation for previously observed discrepancies between results of pressure-denaturation and density gradient centrifugation studies.

W-PM-J9 INDO MOLECULAR ORBITAL CALCULATIONS OF SPIN DENSITIES IN THE DNA BASES.

S.M. Adams* and H.C. Box, Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York 14203.

Molecular orbital calculations at the INDO level of approximation have been employed to obtain the atomic spin densities in the DNA bases. The primary reduction products at 4.2°K, determined from ENDOR experiments for thymidine, uridine 5' phosphate and cytidine 3' phosphate, revealed the main concentration of unpaired spin as C6 in all three cases. These results were confirmed by the INDO calculations. Both INDO and Hückel calculations predict the absence of a resolved ESR doublet splitting in the adenine anion radical. This has been verified by ESR spectra of the radiation damage products. In guanine, the INDO method predicts an oxidation radical with unpaired spin mainly on the oxygen. This species should exhibit a large g shift and large anisotropy. When compared with other theoretical methods, INDO gives good agreement in the treatment of reduction species. There is wide disagreement among the theoretical methods in the calculations for oxidized species.

W-PM-J10 CHARACTERIZATION OF THE CHEMICAL MODIFICATION OF SUPERHELICAL PM2 DNA I WITH CMC CARBODIIMIDE.

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The water soluble N-cyclohexyl-N'-β'(4 methylmorpholinium)-ethyl carbodiimide (CMC) reacts with unpaired thymine and guanine residues under mild conditions to form a covalent product allowing the removal of excess reagent. Consequently this reagent is an excellent probe of nucleic acids suspected of having regions of interrupted secondary structure. Recently it was shown that CMC reacts with SV40 DNA I at specific sites†. In this report we examine the following for PM2 DNA I: 1. The change in $S_{20,w}$ and buoyant density vs. the extent of reaction. 2. The latter was determined from the buoyant density change as well as the specific activity of ^3H CMC in separate experiments. The results are in excellent agreement. 3. Superhelical titrations were performed on molecules early and late in the reaction. The initial reaction of the relatively bulky CMC does not cause a loss of superhelical turns although $S_{20,w}$ increases by 2S units. A 12% decrease in superhelical turns accompanies further reaction with a final increase in $S_{20,w}$ of 6S units. This data can be analyzed from a model of superhelical DNA containing regions of interrupted secondary structure forming partially paired hairpins. The question of the number of these sites in PM2 DNA is under investigation by restriction enzyme mapping.

† See Salzman, N. P., Lebowitz, J., Chen, M., Sebring, E. and Garon, C. F. Cold Spring Harbor Symposium on Quant. Biol. 39 (1975).

W-PM-J11 CHARACTERIZATION OF SPECIAL SITES IN HIGH MOLECULAR WEIGHT CALF THYMUS DNA AT WHICH NON-RANDOM CLEAVAGE OCCURS.

R.S. Welsh and K. Vyska, Institute of Medicine, Atomic Research Center, Jülich, West Germany.

If the conditions during preparation of DNA from calf thymus are so selected that the exposure of chromatin threads to cytoplasmic components and possible enzymatic reactions involving ATP are minimized, the DNA obtained has initially a weight-average molecular weight of 20 million daltons, as determined by the particle length distribution obtained from electron micrographs, and from the mean sedimentation coefficient ($S^{o}_{20,w}$) of 27 S and the intrinsic viscosity at zero shear ($[\eta]$) of 96 dl/g. This DNA can be cleaved as a result of EDTA treatment and subsequent dialysis to DNA subunits having a mean molecular weight of about 500,000, ($S^{o}_{20,w}$=5.9 S, $[\eta]$ = 6.7 dl/g). It could be shown that this effect did not result from endonuclease action or shear degradation, so that it must be assumed that the cleavage reflects some intrinsic property of the DNA. The purity of the high molecular weight DNA used for the reaction was determined to be 0.7% of firmly bound protein, (in agreement with values reported for standard DNA). The final purity after cleavage was about 0.4% of such protein. This difference could be accounted for as the material released during the dialysis, and this material was characterized as phosphopeptides having molecular weights of about 1000 and 1500. Results were obtained showing that the release of the phosphopeptides was in kinetic agreement with the cleavage of the DNA.

W-PM-J12 ELECTRON MICROSCOPIC STUDIES OF NUCLEOTIDE SEQUENCE.

S.D.Rose*, L.G.Marzilli*, C.U.Chang*, M.D.Cole*, B.Hanson*, J.W. Wiggins* and M.Beer. Department of Biophysics and Chemistry, Johns Hopkins University, Baltimore, Maryland 21218.

Electron microscopic studies of base sequence require selective electron-dense markers for bases. All bases, however, must be stained to aid in their enumeration (1). Chloroacetaldehyde reacts selectively with adenine and cytosine moieties. The reaction leaves ØX-174 DNA unbroken, and yields products that react readily with mercuric acetate (2) or osmium tetroxide (3). The contour length of ØX-174 DNA is reduced following reaction of the unmodified DNA with DMSO-Pt Cl_3^- (4). To avoid possible crosslinking, tridentate chelates of Pt(II) have been prepared. The DMSO-$PtCl_3^-$ reagent combines with osmium-reacted nucleotides.

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4. R.F.Whiting and F.P.Ottensmeyer, Proc. 32nd Annual EMSA meeting, 384, (1974).

W-PM-J13 STUDIES ON INTERACTION BETWEEN POLY (LYSINE^{58}, PHENYLALANINE^{42}) AND DNA. R.M. Santella* and H.J. Li, Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, N.Y. 11210.

Complexes of Poly (Lys^{58}, Phe^{42}) and DNA were studied using absorption, circular dichroism (CD) and fluorescence spectroscopy in 0.001M Tris pH6.8. The complexes are soluble until the input ratio r is equal to one lysine per nucleotide. The binding of copolymer to DNA leads to an absorbance enhancement for either DNA or phenylalanine or both, and a fluorescence enhancement for phenylalanine. An increase in fluorescence is also observed with increasing temperature. The binding of copolymer to DNA also induces a red shift and a reduction of the DNA CD above 250nm. The CD change near 220nm is small, however, which is similar to that found with polylysine. Thermal denaturation of the complexes was studied after they were dialyzed to 2.5x 10^{-4}M EDTA, pH8.0. The melting temperature of free base pairs varies from 42-46°C for complexes of varied r value. The bound base pairs show a band at 72-74°C and another broader band about 94°C. The spectral and thermal denaturation results of these complexes support the notion that phenylalanine is at least partially intercalated with DNA after mixing. At higher temperatures more interaction between the phenylalanine chromophore and DNA bases occurs, either through more intercalation into double helical DNA or through more stacking after the base pairs are opened. (supported by National Institutes of Health Grant GM21481.)

W-PM-J14 PLATINUM·DMSO: AN ADENINE-SPECIFIC STAIN FOR DNA SEQUENCING BY ELECTRON MICROSCOPY. R.F. Whiting* and F.P. Ottensmeyer, (Intr.by A.F. Howatson), Department of Medical Biophysics, University of Toronto, Toronto Canada M4X 1K9.

The most promising method for the routine determination of DNA sequences involves the examination of specifically-stained molecules in the electron microscope. DNA molecules are reacted with a stain which binds quantitatively to specific sites and contains heavy atoms. The heavy atoms can be imaged and the sequence determined by the spacing of the marker atoms. Ideally, every base should be stained to show the position of the DNA backbone while an additional heavy atom specifically marks one base type. A platinum complex Pt·DMSO ($KPtCl_3$DMSO) has excellent properties to serve this purpose for sequencing adenine residues. It reacts with two sites on adenine and only one on guanine, cytosine and thymine. On electron micrographs, the doubly-stained adenosine can be distinguished from singly-marked bases in polynucleotides. The reaction is fast and nearly quantitative at 0°C. In a 10-fold excess of platinum the additions are complete in about 50 hours. Poly C combines with slightly more than one Pt/base while poly U reacts with slightly less than one Pt/base. tRNA and double-stranded calf thymus DNA reacted very rapidly with Pt·DMSO to produce heavily-stained molecules by additional mechanisms. Modifications to the basic structure of Pt·DMSO which could improve its usefulness for sequencing will be discussed.

W-PM-K1 DEUTERIUM NMR AND ITS APPLICATIONS TO BIOLOGICAL MEMBRANES. Ian C.P. Smith, G.W. Stockton*, C. Polnaszek*, A.P. Tulloch*, K. Johnson*, F. Hasan*, and L.C. Leitch*, Division of Biological Sciences, National Research Council, Ottawa, Canada K1A OR6.

Deuterium is an inexpensive and convenient alternative to carbon-13 for isotopic labelling and subsequent NMR studies of biological membranes (B.B.R.C. 60, 844 (1974)). The quadrupole moment provides a simple relaxation mechanism and the opportunity to measure order parameters directly via partially-averaged quadrupole splittings. We have labelled a series of fatty acids at different positions and incorporated them at the 2-position of the lyso compound derived from egg phosphatidyl-choline, in addition to insertion of deuteromethyl groups at the amino group of phosphatidylethanolamine. Spectra have been obtained from oriented films, aqueous dispersions, and single bilayer vesicles of these lipids. Relaxation times T_1 and T_2 and quadrupole splittings have been used to derive information on molecular order and mobility, and its dependence on position and the presence of cholesterol. The necessary theoretical formulation of the dependence of the lineshape and relaxation times on molecular mobility and order has been developed. T_1 is determined mainly by rapid intramembrane motion, whereas T_2 depends on the degree of order within the membrane and the rate of overall membrane rotation. Only minor differences are apparent in the organization of single bilayer and multibilayer vesicles, in contrast to the conclusions reached from ^{1}H NMR. Cholesterol serves to increase the degree of molecular order in the fatty acid chains with very little effect on their mobility. The influence of cholesterol is much greater at position 12 than at position 18. Acholeplasma laidlawii incorporates stearic acid labelled at position 18 into its membranes to yield useful ^{2}H NMR spectra.

W-PM-K2 HIGH-RESOLUTION NEUTRON DIFFRACTION STUDIES OF LECITHIN AND LECITHIN-CHOLESTEROL LIPID BILAYERS. J. K. Blasie, G. Zaccai* and B. Schoenborn (Intr. by A. Horwitz). Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174 and Department of Biology, Brookhaven National Laboratory, Upton, New York 11973.

The atomic scattering factors for X-rays and neutrons are of different origin. Thus, the independently derived X-ray structure and neutron structure for a particular membrane system provide independent tests for the interpretation of these derived structures in terms of molecular and atomic distributions within the membrane. The X-ray scattering density (or electron density) profile for a membrane can be determined from lamellar X-ray diffraction from hydrated oriented multilayers of the membrane using relatively direct methods. The neutron scattering density profile for the membrane can be determined from lamellar neutron diffraction from the same membrane multilayer via a difference Patterson function analysis utilizing the large differences in neutron scattering factors for protons and deuterons. Lamellar neutron diffraction data from dipalmitoyl lecithin multilayers and dipalmitoyl lecithin:cholesterol (2:1 molar ratio) multilayers hydrated with variable H_2O:D_2O mixtures were collected at the High Flux Beam Reactor at Brookhaven National Laboratory. A difference Patterson function analysis determined the neutron scattering profile structures including the bound water profile structures for the two bilayer membranes at $\sim$5 Å resolution. The interpretation of these profile structures together with their previously determined X-ray profile structures in terms of the locations and conformations of the molecular components in the bilayer membranes will be presented.

W-PM-K3 SPIN-LABELS AS PROBES FOR IONOPHORE AND LIPID-SOLUBLE ION INTERACTIONS WITH LIPOSOMES. H. Dugas, N. G. Seidah*, G. Roy, R. Laprade and T. Cyr*, Department of Chemistry and Physics, University of Montreal, Montreal, Que. H3C 3V1.

Electrical conductivity and EPR spin-label methods have been used to study the effect of the ionophores valinomycin, dinactin and alamethicin and the lipid-soluble anion tetraphenylboron (TFB) on molecular organization at the surface and at various depths from the surface of phosphatidylcholine (PC) black lipid membranes (BLM) and liposomes. Stearamide, cholestane and amphiphilic fatty esters spin-labels were used. No structural perturbations either at the surface or the interior of PC liposomes could be detected with spin-labels for as much as 10 mole % of ionophores. However, for a given temperature and pH, the mobility of the spin-labels is reduced when the concentration of the lipid-soluble anion TFB is increased. The mobility of the labels approaches a limiting value at [TFB] $\geq$ 4mM and the membrane is said to be saturated. When cholesterol is added to the membrane, the saturation value is reduced by one-half. The conductance measurements showed that the PC membranes posses a net positive charge for pH $\leq$ 5 and a net neutral charge for pH 6 to 9. It is concluded that TFB adsorbs on the membrane surface and causes the largest perturbation there. For TFB, conductance and spin-labeling results agree quite well.

W-PM-K4 PARAMAGNETIC QUENCHING OF PERYLENE FLUORESCENCE IN LECITHIN AND LECITHIN-CHOLESTEROL LIPOSOMES BY SPIN-LABELLED LIPID ANALOGUES. V.G. Bieri* and D.F.H. Wallach, Radiobiol.Div., Tufts New England Medical Center, 136 Harrison Ave., Boston, Mass. 02111, USA.

We have measured the fluorescence of perylene incorporated into dipalmitoyllecithin (I) and cholesterol-dipalmitoyllecithin (II) (molar ratio 1:1) liposomes. In both lipid matrices we observe identical fluorescence spectra but, at the phospholipid phase transition (41°C) fluorescence increases sharply in system (I); no such fluorescence jump is manifest in system (II). Perylene fluorescence is quenched by all paramagnetic lipid analogues used in this investigation; highest quenching activity is effected by cholestane-nitroxide (CSL) followed by androstan-nitroxide (ASL), 5-nitroxide-stearate (5NS) and 16-nitroxide-stearate (16NS). However, fluorescence quenching in (II) is markedly reduced compared to that in (I). Fluorescence quenching in (I) is stable with temperature below 35°C and above 45°C, but decreases sharply about 41°C. In the presence of 50 mol % cholesterol fluorescence quenching diminishes linearly, but only slightly between 25-60°C. CSL and ASL at concentrations >20 mol % themselves suppress the quenching discontinuity at the phase transition; this indicates that these sterol spin-labels simulate cholesterol in its structural effects. The quenching phenomena observed are attributed to a non-random accomodation of fluorophore and quencher molecules (co-clustering) below the phase transition and a statistical distribution of both impurities above 41°C. In the presence of cholesterol the clustering tendencies are reduced. This is compatible with the concept that cholesterol fluidizes the phosphatide acyl chains below the transition temperature.

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W-PM-K5 EFFECT OF IONS AND CHOLESTEROL ON THE HUMAN ERYTHROCYTE LIPID-WATER LAMELLAR PHASE. E. Izaguirre* and Y. Lange, Biophysical Laboratory, Harvard Medical School, and G.G. Shipley, Biophysics Division, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts.

Using x-ray diffraction techniques, mixtures of erythrocyte lipids and water have been shown to exist as a single lamellar phase in the range of 28-75% water at 20°C, in agreement with the work of Rand and Luzzati (Biophysical J. (1968) 8, 125). The repear distance, d, increased from 69Å at 28% water to 200Å at 75% water. When the mixtures were prepared in a .1M solution of equimolar amounts of NaCl, RbCl and CsCl, the repeat distance, above 40% water, was smaller than in the case of deionized water for all water contents. These results indicate the extrusion of ions and water from the interlamellar space due to the presence of charged phospholipids. When $CaCl_2$ was added at molar ratios Ca^{++}/phospholipids above .06 ± .02 the repeat distance was 70.9 ± 1.70Å, independent of water content in the range from 28-70% water. These results suggest that the Ca^{++} binding sites relevant to the swelling behavior are associated with phosphatidylserine and phosphatidic acid, present in the lipid mixture at the molar ratio of .15 ± .01, and that the binding ratio Ca^{++}/acidic phospholipid is 1:2. When cholesterol was removed from the lipids, the bilayer thickness decreased from 44 to 39Å and the surface area per phospholipid polar head group increased from 50 to 64Å^2. In the absence of cholesterol the wide angle reflection remained broad and was centered at 4.41 ± .04Å in comparison with the value of 4.67 ± .05Å obtained in the total lipid mixtures. The step-wise readdition of cholesterol to the phospholipid fraction gradually reversed this change. Supported by USPHS grants 3 R01 HL13513-03 and AM11453.

W-PM-K6 PHYSICAL STATE OF THE MULTICOMPONENT SYSTEM CONTAINING EGG YOLK LECITHIN, CHOLESTERYL LINOLEATE, AND WATER. C. R. Loomis, G. G. Shipley, and D. M. Small, Biophysics Division, Boston University School of Medicine, Boston, Mass. 02118

Polarizing light microscopy, differential scanning calorimetry (DSC), and x-ray diffraction were used to define the temperature-composition ternary phase diagram of egg yolk lecithin (EYL), cholesteryl linoleate (CL), and water. A single phase zone exists at 23°C which incorporates CL up to a maximum molar ratio of 1 CL:18 EYL at 23 weight % (wt.%) water. Increasing the water concentration above 23 wt.% causes a decrease in incorporated CL with total exclusion by 40 wt.% water. Observation of this single phase using the polarizing microscope demonstrates a fluid, smectic liquid-crystalline phase giving a positive sign of birefringence. X-ray diffraction of the single phase gave several low angle reflections in the ratio 1:1/2:1/3:1/4...and a diffuse band centered at 4.6Å, indicating a lamellar structure with the hydrocarbon chains in a fluid or disordered state. The influence of CL upon the lamellar phase is demonstrated by comparison of the lamellar repeat distance d, for EYL and the mixed lipid system. At 30 wt.% water, d (EYL) of 56.2Å is decreased at maximum CL incorporation to 55.4Å. The calculated thickness of the hydrocarbon layer decreases from 39.4 to 38.6Å and the mean surface area/molecule increases from 63.6 to 64.5Å^2. Below 40% water, mixtures containing CL in excess of 1 CL:18 EYL separate into two phases; CL saturated lamellar liquid crystal of EYL and pure crystalline CL. In mixtures containing greater than 40 wt.% water, three phases coexist; EYL liquid-crystal, crystalline CL and water. Although CL melts at 42.5°C, no discernible change in phase boundaries was observed between 0 and 60°C. The low solubility of CL in EYL may explain why little cholesterol ester is found in biological membranes.

W-PM-K7 PHASE ANGLE DETERMINATION OF X-RAY REFLECTIONS FROM SYMMETRIC STRUCTURES: A NEW TECHNIQUE APPLIED TO MEMBRANES. J. B. Stamatoff and S. Krimm, Department of Physics, Univ. of Michigan, Ann Arbor, Mich. 48104.

Phase angles of x-ray reflections have been determined for biological membranes by analysis of data obtained for fluid layers of variable thickness. These data are invaluable for phase angle determination of higher resolution diffraction patterns in which the small fluid layer thicknesses, usually required to eliminate disorder, preclude the application of direct deconvolution methods. Previous methods of analysis are sensitive to scaling factors between sets of reflections and the value of the origin reflection. In addition, variation of the intermembrane fluid density from one set of reflections to the next is prohibited.

In a new technique, phase angles are computed from the observed data without using approximated scaling factors or origin reflection values. Electron density functions obtained from two different sets of observed reflections which are assigned two sets of phase angles may be envisioned as being plotted, one against the other. The correct choice of phase angles will produce a straight line whose slope is the scaling factor and whose intercept is related to the origin reflection. Least mean square deviations from a straight line are used to determine the excellence of the fit, thus eliminating the need to display the plots. The deviations, scaling factors, and origin reflections have been calculated in closed form as a function of the observed reflections permitting rapid computer computation of each quantity for all possible combinations of phase angles. The calculation may be performed over the intramembrane region thereby allowing variable intermembrane fluid densities. The results of the application of this method to several model structures, myelin data, and erythrocyte data will be discussed.

W-PM-K8 THE SURFACE DISTRIBUTION OF THE FATTY ACID SIDE CHAINS OF PHOSPHATIDYLETHANOLAMINE IN MIXED PHOSPHOLIPID VESICLES. Burton J. Litman, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22903.

Mixed phosphatidylethanolamine (PE)-phosphatidylcholine (PC) sonicated vesicles have been employed to study the distribution of fatty acids associated with the PE molecules located in the inner and outer vesicle surface. Selective labeling of the outer surface PE molecules by reaction with the membrane impermeable reagent 2,4,6-trinitrobenzenesulfonic acid (1), followed by separation of the labeled and unlabeled PE by thin layer chromatography, effects a separation of the inner and outer surface PE molecules. Subsequent methyl ester formation and GLC analysis allows the determination of the mole percent distribution of fatty acid side chains associated with these two fractions. Measurements on unlabeled vesicles provide similar information concerning the total PE incorporated into the vesicle. Our results show that the fatty acid side chain distributions observed in the PE fractions derived from the inner and outer vesicle surfaces are the same as that of the PE stock solution from which the vesicles were prepared. Similar data were obtained for the side chain distribution of the total PE in the vesicle. These results support the contention that the head group packing is the major determinant of the surface distribution of the PE molecules in these systems and demonstrate that the fatty acid side chains associated with these molecules have the same mole percent occupancy in the two surfaces of the vesicle.
(Supported by NSF Grant GB-41313).

1. Litman, B.J. (1973) Biochem. 12, 2545.

W-PM-K9 CELL FUSION IN ARTIFICIAL SPHERICAL BILAYER MEMBRANES
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Spherical bilayer membranes composed of the naturally occurring phospholipids, phosphatidylcholine (PC) and phosphatidylserine (PS) as well as some synthetic phospholipids were used to study the phenomena of cell fusion as a function of temperature. Fusion was also studied as a function of temperature by varying a number of other parameters, which included the distribution of mono and divalent cations, and the addition of lysolecithin and cholesterol to the membrane phase. These experiments indicated a temperature dependent nature for the fusion process, but more importantly they demonstrated the need for the membrane to be fluid as a necessary condition for fusion to occur. The possibility for occurrente of the membrane fusion is related to the degree of fluidity of the membrane. These results were correlated with monolayer dissolution studies and conductance studies on bilayer membranes in order to propose a mechanism for the fusion process in an artificial membrane system.

W-PM-K10 ELECTRON DIFFRACTION INVESTIGATION OF HEXAGONAL ALIPHATIC CHAIN PACKING IN CRYSTALLINE LIPIDS.
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The existence of a hexagonal methylene subcell for aliphatic chain packing has been observed to be of importance in the phase behavior of membrane lipids. This packing has been interpreted in terms of models such as proposed by Vainshtein (Sov. Phys. - Crystallogr. 2 (1957) 334), but, due to the difficulty in growing suitable large crystals for X-ray diffraction experiments, quantitative corroborative intensity data have been unavailable. Taking advantage of the enhanced scattering cross-section of matter for electrons, single crystal transmission electron diffraction patterns have been obtained from thin microcrystals of *rac* glycerol 1,2-dipalmitate, 1,2-dimyristoyl-*sn*-glycerophosphoryl choline, N-nervonoyl-sphingosyl-1-phosphoryl choline and a mixture of 1-palmitoyl- and 1-stearoyl-*sn* glycerophosphoryl cholines at 80kV. The highest resolution data were observed in diffraction patterns from the 1,2-diglyceride and the lecithin, giving 5 unique hk0 intensity data (plane group p6m) to a resolution of $d^*=0.723\text{Å}^{-1}$. In all cases the unit cell constants are a=b=4.80Å, $\gamma=120°$. The intensity data from all compounds conform very well to interpretation by the kinematical theory and support the cited rigid methylene rotor phasing model for the projection.

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W-PM-K11 KINETIC ASPECTS OF THE GEL-LIQUID CRYSTAL TRANSITION IN PHOSPHOLIPIDS.* B. W. Maxfield, R. M. Clegg*, L. Avery* and E. L. Elson*. Department of Chemistry, Cornell University, Ithaca, New York 14853.

A newly developed kinetic method employing small periodic pressure changes to perturb the equilibrium state has been used to study the dynamic aspects of the gel-liquid crystal transition in synthetic phospholipid vesicles. Pure dimyristoyl-L-α lecithin (DML) and mixed DML-dipalmitoyl-L-α lecithin sonicated vesicles have been studied. The characteristic time and amplitude response induced by 3 atmosphere step changes in pressure were monitored by changes in turbidity. Measurements were made at many temperatures within the narrow transition region (determined from equilibrium measurements of turbidity vs. temperature). As in previous temperature jump measurements (T. Y. Tsong, PNAS, 71, 2684 (1974)), at least two characteristic times are evident in the kinetic data. The amplitude associated with each time increases from a negligibly small value below the melting temperature, T_M, to a maximum within the transition region and then decreases at higher temperatures. The same qualitative behavior is observed for the temperature dependence of each characteristic time. Interpretation of these results requires determining the origin of the turbidity change. Studies of more controlled and characterized vesicle preparations are in progress. We expect that these studies will yield information about the properties of various phases that can coexist in equilibrium in membranes.

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W-PM-K12 LIPID BILAYER ULTRASTRUCTURE IN THE NATURAL STATE.
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Single lipid bilayers and associated cations have been electron microscopically visualized in the absence of preparative staining, embedding, freezing etc. Phospholipids of fatty acid (FA) salts were collected from the air/water interface as either oriented Langmuir--Blodgett multilayers or as collapsed monolayers. Pure FAcid multilayers were found to lack individual bilayer contrast whereas FA salts appear highly lamellar. Divalent metal salts introduced headgroup contrast in the sequence Ba> Sr> Ca> Mg. Monovalent FA salt multilayers appear distinctly lamellar with headgroup contrast decreasing in the order Cs> Rb> K> Na> Li. Increasing the FA chain length from 14 to 30 carbons resulted in a corresponding increase in bilayer thickness. Bilayers of the Ba salt of the 22 carbon acid were found to be 53 A thick as determined by both EM and x-ray methods. Multilayers of saturated lecithin lacked lamellar appearance in natural and stained preparations whereas phosphatidylserine multilayers developed strong bilayer densities upon treatment with Os. Face view images through unsupported single lipid bilayers have been recorded. Electron diffraction patterns were absent from single bilayers except in film defect zones. The hexagonal diffraction patterns of the defects were indistinguishable from electron diffraction patterns of single phospholipid crystals.

W-PM-L1 FLUIDITY IN CELL MEMBRANES: MOLECULAR MODELING OF THE PARTICLE AGGREGATION SEEN BY ELECTRON MICROSCOPY

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The freeze-cleave-etch technique has shown that intermembranous particles of plasma membranes can congregate in various degrees and forms of aggregation. In the present study, the fluid mosaic model has been simulated by assuming that 8.5 nm diameter particles (at the same density observed in electron micrographs):-(i) are free to move in two dimensions in a medium of given viscosity (ii) are allowed to move in steps of a fraction to a few microseconds duration by a simple diffusion process and (iii) interact when two particles approach each other closely enough. The particles are not confined to movement in a grid. The process has been followed up to times of the order of 50,000 microseconds, and sequential pictures made of the positions of the particles. The resulting simulated electron micrographs are similar to published freeze-cleave electron micrographs of different stages of aggregation of particles. Hence, since the assumptions made in the simulation are few and relatively well understood, the fluid mosaic model (S.J.Singer and G.L.Nicolson, Science (1972) 175 720) is sufficient to describe the observed aggregation phenomena. Further, the simulation model, with data from the observed electron micrographs, then gives information about the range of the interparticle interactions, etc. It also appears that, for a reasonable degree of aggregation, only a certain range of particle density is allowed--which may be related to biological function.

+ N.I.H. Special Fellow and American Cancer Society Research Scholar at U.C.S.D. Programming was done by Jon H. Shirley; Computer funds: U. of CO.

W-PM-L2 FLUIDITY OF NATURAL AND ARTIFICIAL MEMBRANES: EFFECTS OF LOCAL ANESTHETICS AND CHOLESTEROL.

S.M. Fernandez*, M.B. Feinstein* and R. I. Sha'afi. The University of Connecticut Health Center, Farmington, Connecticut, 06032.

The dynamic behavior of the hydrocarbon region of natural and artificial membranes was investigated by a combination of steady-state fluorescence polarization and nanosecond relaxation techniques employing perylene as a hydrophobic fluorescent probe. The systems studied include phosphatidyl serine and ganglioside liposomes; myelin, erythrocyte, PMN-leukocyte, and mitochondrial membranes, and intact living ascites cells. Quantitative measurements of the microviscosity ($\bar{\eta}$) in the interior of these membranes were obtained as a function of temperature (8°-38°C). In addition, the effect of incorporation of cholesterol and local anesthetics on membrane fluidity was investigated. Plots of ln $\bar{\eta}$ vs 1/T were found to be linear for the artificial liposomes and non-linear for the natural membranes studied, indicating partial phase changes in the latter case. Significant differences were also observed in the fluidity of the various natural membranes studied. The highest $\bar{\eta}$ seen in PMN-leukocytes (330 cps), followed by myelin (270 cps), human erythrocyte (180 cps) and mitochondrial membranes (90 cps). These differences may reflect structural variations necessary for the underlying functional requirements of each membrane type. Incorporation of cholesterol resulted in an increase in microviscosity and a decrease in entropy in all systems studied, which suggests immobilization of the hydrocarbon chains by the cholesterol. The local anesthetic tetracaine produced a decrease in microviscosity in purely lipid membranes, isolated natural membranes, and intact ascites tumor cells. This decrease may result from an expansion of membrane area due to insertion of anesthetic molecules into the lipid bilayer. Supported by GM-17536 and GM-20268.

W-PM-L3 MOTION OF IMPURITY MOLECULES IN SOLID UNSATURATED LIPIDS. G.E. Cohn and L.I. Grossweiner*, Biophysics Laboratory, Physics Department, Illinois Institute of Technology, Chicago, Illinois 60616.

ESR of nitroxide spin label probes has been employed to investigate the translational diffusion and motional freedom of impurities in a solid lipid environment. These systems offer a simple analog to the behavior of foreign molecules within the ordered hydrocarbon zone of a biological membrane. Pooling into regions of high local spin label concentration can be detected from changes in line shape characteristic of increased interactions between labels, while rotational motion can be determined from measured spectral parameters. Measurements performed on the monoenoic lipid oleic acid with a saturated hydrocarbon spin label at temperatures above and below the melting point indicate that rotational motion decreases with an Arrhenius activation energy of 6-7 kcal/mole but does not change drastically as the matrix temperature is decreased slowly to below the freezing point. However, a distinct alteration in lineshape occurs at 5°-10° below freezing which indicates an increase in interactions between labels characteristic of pooling. Spectra obtained following rapid quenching and slow incremental heating exhibit a different temperature dependence in the bulk solid matrix, with significant nitroxide-nitroxide interactions evident closer to the melting point than for slow cooling. The motional behavior of spin labeled impurities in the unsaturated matrix differs qualitatively from their behavior in a saturated matrix, giving an indication of the effect of double bonds on translational and rotational diffusion. (Supported by U.S. A.E.C. Contract AT(11-1)-2217.)

W-PM-L4 THE DETECTION OF STRUCTURAL DIFFERENCES BETWEEN THE MEMBRANES OF NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED CHICK EMBRYO FIBROBLASTS BY SPIN LABELING. T. Buckman, M.J. Weber,* and T.M. Yau,* Dept. of Microbiology, Univ. of Illinois, Urbana, Ill. 61801.

Stearic acid derivatives with nitroxide substituents at various positions of the hydrocarbon chain were incorporated into normal growing, density-inhibited or Rous sarcoma virus-transformed cultures of chick embryo fibroblasts. The results indicate a small decrease in membrane fluidity in the transformed cells and a small increase in membrane fluidity in the density-inhibited cells relative to the normal growing cells. Stearic acid molecules labeled in the 16 position showed the largest differences in motional freedom of the label and molecules labeled in the 5 position showed the smallest differences. Qualitatively similar results were obtained with whole cells and with isolated plasma membranes, and with cells infected by a temperature-conditional transforming mutant of Rous sarcoma virus. The results correlate well with observed variations in lipid fatty acid compositions, since the transformed cell lipids display a decreased percentage of arachidonic acid relative to that seen in normal cell lipids.

W-PM-L5 ION-INDUCED ORGANIZATIONAL CHANGES IN THE SPIN-LABELED GAR FISH OLFACTORY NERVE MEMBRANE. Bruce W. Stavens* and F.P.J. Diecke* (Intr. by C.C. Wunder), Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242.

The effect of external ions on the physical state of membrane lipids has been studied by electron spin resonance in the spin-labeled gar fish olfactory nerve. Organizational changes induced by variations in $LaCl_3$, $CaCl_2$ and NaCl concentrations were monitored using the 4', 4' dimethyl-oxazolidine analog of 5α-androstan-17β-01-3-one. The degree of organization of the membrane lipids was found to be dependent upon the concentration and valence of the cations in solution bathing the nerve membrane. Plots of the order parameter h_0/h_{-1} versus concentration for the three cations over the range 0-100mM exhibit saturation for La^{+++} and Ca^{++} but not for Na^+. These results suggest that the external cation concentration is an important factor in determining the physical state of membrane lipids. The observed changes in ordering of membrane lipids may be interpreted in terms of changes in the electrostatic interactions between phospholipid polar head groups. Qualitatively, the relative effects of La^{+++}, Ca^{++} and Na^+ conform to the predictions of diffuse double layer theory. Supported by NIH Grant NS 05188.

W-PM-L6 PHASE TRANSITION OF HEPATOCYTE AND HEPATOMA PLASMA MEMBRANES BY ELECTRON DIFFRACTION. S. W. Hui, D. F. Parsons and N. Schulman*. Roswell Park Memorial Institute, Electron Optics Lab., Buffalo, N.Y. 14203

Plasma membranes from normal rat hepatocytes and from transplanted Reuber Hepatoma cells were purified by the sucrose gradient method (Emmelot et al., BBA 90, 126 (1964)) as well as by the two phase polymer method (Lesko et al., BBA 311, 173 (1973)). Purity was monitored by electron microscopy and by enzyme marker assays. The morphology, relative purity and enzyme activities of the specimens will be discussed. Freshly prepared wet membranes were studied by electron diffraction, using a hydration state designed for a Siemens Elmiskop IA (Hui et al., Proc. 31st EMSA 340 (1973)). A faint but sharp diffraction ring with spacing 4.1Å was seen for all specimens at temperatures below 10°C. A diffuse band with spacing about 4.6Å was also observed. The appearance of the two rings suggests the existance of phase separation in these membranes. The sharp ring disappears at higher temperature, indicating that portions of the membrane have undergone a phase transition from an ordered to a less ordered state. The transition temperature is $18^0 \pm 1^oC$, for hepatoma plasma membranes. For normal hepatocyte plasma membrane the transition temperature range is very wide ($15^0 \pm 5^oC$) The significance of the difference will be discussed in the context of lipid composition, membrane fluidity and neoplastic transformation. Parallel freeze fracturing study will be presented. This work is supported by an NCI Grant CA 15330.

W-PM-L7 CORRELATION BETWEEN LIPID CHAIN MOBILITY AND CALCIUM ATPASE IN SARCOPLASMIC RETICULUM MEMBRANE AFTER REPLACEMENT OF PHOSPHOLIPIDS.
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Analysis of the kinetics of the Ca^{2+}-ATPase of fragmented sarcoplasmic reticulum (FSR) shows that replacement of the endogenous phospholipids by dipalmitoyl lecithin (DPL) or dioleyl lecithin (DOL) (Warren et al., P.N.A.S. 71, 622,1974) profoundly affects k_2 in the following scheme:

$$E + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \sim P + ADP \xrightarrow{k_2} E + P_i,$$

while having little or no effect on k_1 and k_{-1}. At 0° k_2 of DPL-SR is 50 times less than that of control FSR, while the value for DOL-SR is half that of native FSR. As the temperature is raised the difference between native FSR and FSR in which the lipids have been replaced decreases. Electron spin resonance spectra of a fatty acid spin probe incorporated into these different SR systems show that the mobility of the label is a function both of temperature and of the nature of the phospholipid molecules present. The temperature dependence of the mobility of the spin label in the various systems parallels the effect of temperature on the decomposition of the E~P intermediate. These data clarify previous reports dealing with the role of lipids in the ATPase activity of FSR in that they suggest a correlation between the fluidity of the lipid moiety and its effect on the E~P decomposition step.
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W-PM-L8 REDUCTION OF SPIN PROBES BY MITOCHONDRIA AND MICROSOMES AND ITS USE TO MONITOR TRANSFER OF SPIN-LABELLED LIPIDS BETWEEN CELL ORGANELLES- S.Schreier*, L.Stuhne-Sekalec* and N.Z.Stanacev*, Institute of Chemistry, Universidade de São Paulo,Brazil and Dept. of Clinical Biochemistry, Banting Institute, University of Toronto, Canada, Intr. by A. Ehrenberg.

The mechanism of transfer of lipids between cell organelles is an open question. We have investigated this problem with the spin label method, making use of the intrinsic ability of mitochondria and microsomes to reduce the mitoxide moiety. The spin probes used were either uptaken in the lipid regions of the membranes,or bound to membrane proteins. A water-soluble probe was also employed. The esr signal of the probes was destroyed in the presence of the biological material. The rate of reaction was faind to depend on the composition of the suspending medium, on the nature of the probe, on probe, concentration and on the nature of the biological material. Both the protein bound probes and those intercalated in the lipid regions of the membrane were reduced at similar rates. When the probes were intercalated in phospholipid vesicles, destruction of the esr signal occurred when the vesicles were mixed with the sub cellular fractions. These results, in connection with experiments on the reduction of probes which were first uptaken by microsomes and further exposed to mitochondria indicated that transfer of probes from the former to the latter took place. - This work was supported by grants from J.P.Bickell Foundation the Ontario Heart Foundation, and the Medical Research Council of Canada.

W-PM-L9 ASYMMETRY OF THE BILAYER OF INFLUENZA VIRUS MEMBRANE Kuang-Hsin Tsai* and John Lenard, Department of Physiology, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854

Enveloped viruses form by budding from the plasma membrane of their host cell, incorporating host plasma membrane lipids. Viral particles offer advantages similar to those of the red cell for the study of membrane asymmetry: (1) only the outer surface is exposed to the external environment; (2) contains no internal membrane structures; (3) can be readily re-purified after side-specific modification so that only structurally intact virions are analyzed. We have hydrolyzed intact and spikeless particles of influenza grown in MDBK cells using phospholipase C of two specificities--from Cl. Welchii which does not hydrolyze PS, and from B. Cereus which does not hydrolyze Sph. Both enzymes hydrolyzed over 80% of PC, but only 43% of PE from intact virions. The Cl. Welchii enzyme hydrolyzed 51% of Sph from intact and 71% from spikeless particles, while the B. Cereus enzyme hydrolyzed only 37% of PS. The variability of the phospholipid composition of MDBK-grown influenza and the implications for virus bilayer asymmetry will also be discussed. These results suggest that the viral bilayer has similar asymmetry to the red cell membrane, and it is reasonable that this reflects the arrangement in the host cell membrane also. Supported by NSF grant #GB-36789.

W-PM-L10 A DIRECT ANALYSIS OF LAMELLAR X-RAY DIFFRACTION FROM LATTICE DISORDERED RETINAL RECEPTOR DISK MEMBRANE MULTILAYERS AT 8 Å RESOLUTION. G. Santillan* and J. K. Blasie (Intr. by T. Wood). Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174.

Oriented membrane multilayers containing a membrane pair within the unit cell potentially possess both lattice disorder and substitution disorder. A simple form of substitution disorder can arise from a variation of the separation of the two membranes within the pair among unit cells in the multilayer. Simple substitution disorder produces an incoherent diffuse scattering underlying the lamellar diffraction which, in the presence of lattice disorder, can prevent the direct determination of a unique high-resolution unit cell electron density profile from the lamellar diffraction. This is the case for lamellar diffraction from intact retinal rods. The generalized Patterson function analysis for the lamellar diffraction from lattice-disordered multilayers permits the identification of the autocorrelation function and the continuous structure factor modulus of the unit cell electron density profile. Both functions are unique to the profile and therefore can be used to derive directly the membrane pair profile if the profile projection possesses a center of symmetry. Removal of non-covalently bound mucopolysaccharides from the isolated retinal disk membranes prior to formation of the multilayer significantly reduces both lattice and particularly substitution disorder in the multilayer. This procedure has permitted the direct determination of the disk membrane electron density profile at 8 Å resolution in either the unbleached or bleached states of the membrane. The majority of the scattering mass of rhodopsin is easily identified in such high resolution profiles and the distribution of this mass changes dramatically upon rhodopsin bleaching.

W-PM-L11 A DIRECT ANALYSIS OF LAMELLAR X-RAY DIFFRACTION FROM LATTICE DISORDERED MULTILAYERS OF FULLY-FUNCTIONAL SARCOPLASMIC RETICULUM MEMBRANES AT 10 Å RESOLUTION. J. Marquardt*, A. Scarpa and J. K. Blasie. Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174.

Highly hydrated (>60% water) oriented multilayers of rabbit skeletal muscle sarcoplasmic reticulum vesicles which were relatively homogeneous in their density distribution were formed by mild sedimentation and subsequent slow partial dehydration at 4°C. These oriented multilayers are themselves capable of ATP-induced Ca^{++}-uptake as are the resuspended vesicles as monitored spectrophotometrically using the Ca^{++} indicator arsenazo III. Lamellar X-ray diffraction from such functional multilayers shows monotonically increasing widths for higher order lamellar "reflections" indicative of simple lattice disorder within the multilayer even when the data is obtained in a few minutes using a position sensitive X-ray detector. A generalized Patterson function analysis developed for treating lamellar diffraction from lattice disordered multilayers allows the identification of the autocorrelation function of the unit cell electron density profile and its subsequent deconvolution to provide directly the unit cell membrane pair profile which possesses a center of symmetry in the profile projection. This analysis allows the identification of a more precise extent of the membrane pair profile. The resulting sarcoplasmic reticulum membrane profile possesses marked asymmetry both with regard to relative electron density levels and distributions on either side of the membrane. Our results will be compared with the previous structural work of others on this membrane.

W-PM-L12 FREEZE-FRACTURE PARTICLES IN PROTEASE TREATED MEMBRANES. D.W. Deamer and N. Yamanaka*, Dept. of Zoology, Univ. of Calif., Davis. 95616.

The origin of the particulate structures demonstrated in membranes by freeze-fracture electron microscopy is an important problem, since the particles may be related to lipid-protein organization within the plane of the membrane. To investigate this problem, we have treated several membrane systems with Nagarse, a bacterial protease, and followed biochemical and ultrastructural properties of the membranes during digestion. The membranes chosen were sarcoplasmic reticulum (SR), rat liver submitochondrial fragments enriched in inner membrane (SMP) and human erythrocyte ghost membranes. Protease/membrane protein ratios varied from 1:2 to $1:10^4$, and digestion time from 0 to 60 min at 25°. At ratios of 1:10 or greater, digestion of all three membranes was complete in 10-15 min with about half the original protein remaining in the membrane. Several enzyme activities, including the ATPase of SR and the ATPase and succinic oxidase of SMP, were lost during the same interval. SDS-gel electrophoresis showed that all original polypeptide bands were absent in digested membranes, and were replaced by a diffuse band of 10-15,000 M.W. When SMP was compared by freeze-fracture microscopy before and after digestion, little change was found in particle size or number. In SR and ghost membranes, the number of particles per unit area decreased by about half, but particle size was unchanged. In all three membranes particle distribution between inner and outer fracture faces was more symmetric following digestion. We conclude that high molecular weight polypeptides are not required for particles to be produced in membrane fracture faces. Low molecular weight polypeptides, either as aggregates or as lipoprotein complexes, are sufficient to produce particles in membranes visualized by freeze-fracture microscopy.

W-PM-L13 PHYSICOCHEMICAL PROPERTIES OF p-Cl-ANILINONAPHTHALENE-6-SULFONATE (p-Cl-ANS) AND MEROCYANINE-540 (M-540) IN VITRO AND IN NERVE MEMBRANES: DEMONSTRATION OF ENERGY TRANSFER. I. Tasaki, A. Warashina* and H. Pant, Lab of Neurobiology, Natl. Institute of Mental Health, Bethesda, Md. 20014.

Absorption and fluorescence emission spectra of p-Cl-ANS and M-540 were examined in vitro. The absorption band (of the lowest energy) of p-Cl-ANS was about 360 nm and the wavelength of maximum emission was 430 nm in ethylene-glycol and 407 nm in ethanol. Beer's law was not obeyed by M-540 dissolved in water or in chloroform, indicating the presence of dimers in these media. The fluorescence yield of dimers in chloroform was extremely low. Addition of a small amount of ethanol to an M-540 chloroform solution converted dimers into monomers. The behavior of these probes in various macromolecules and in the nerve membrane was examined. Fluorescence signals (associated with action potentials) observed in nerves stained with p-Cl-ANS were attributed to a change in the polarity of the microenvironment of the probe molecules in the membrane. A reversal of the sign (at about 550 nm) of absorption signals was demonstrated in crab nerve stained externally with M-540, indicating the presence of dimer-to-monomer conversion during nerve excitation. The excitation spectrum of M-540 in the nerve membrane was determined in both the resting and the active state of the nerve. By incorporating both p-Cl-ANS and M-540 into a crab nerve or a squid giant axon, it was possible to demonstrate resonance transfer of energy from p-Cl-ANS (excited at 360 nm) to M-540 (detected at about 620 nm). Photic energy absorbed by p-Cl-ANS molecules in the external layer of a squid axon membrane could be transferred to M-540 in the inner layer of the axon. It was also possible to record fluorescence signals mediated by energy transfer. The significance of these findings will be discussed.

W-PM-M1 INFLUENCE OF ION-PAIR FORMATION REACTIONS ON FLUX OF AN INERT SPECIES ACROSS A MEMBRANE. V.S.Vaidhyanathan, Dept.Biophysics State University of New York at Buffalo.

Choosing a well-specified example, of a membrane diffusion barrier, of three univalent ions undergoing transport, the reaction rates, concentration profiles and electrical potential profiles are computed, when ion-pair formation reactions occur in the membrane phase, for specified boundary concentrations and rate constants. The influence of such chemical reactions on the magnitude and direction of flux of a neutral species (with its specified boundary concentrations), which does not participate in the chemical reactions is computed a. using Fick's law, b. using partial frictional coefficient formalism, with assumed values of diffusion coefficients at one interface, when there is no chemical reaction and electrical potential gradient vanish, c. when there is chemical reaction and electrical potential gradient vanish, d. when electrical potential gradient does not vanish. Numerical results of such calculations are presented.

W-PM-M2 A MACROSCOPIC DEVELOPMENT OF THE USSING FLUX-RATIO EQUATION. David C. Dawson, Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242.

Ussing (Acta Physiol. Scand., 1949, 19:43-56) developed the flux-ratio equation by describing ionic diffusion locally via the Nernst-Planck equations, and integrating to obtain a global relation for the ratio of unidirectional fluxes as a function only of the concentrations (C_1,C_2) and electrical potential (ψ) on the two sides of a membrane. It is possible to develop this relation from macroscopic considerations alone by noting that the ratio of unidirectional rate coefficients for a freely diffusible substance at equilibrium is given by:

$$\{K_{12}/K_{21}\}_{eq} = \exp\{\frac{ZF}{RT}\Delta\psi_{eq}\} \qquad (T,P \text{ constant})$$

In addition, macroscopic postulates for ideal tracer behavior dictate that:

$$K_{12}/K_{21} = K^*_{12}/K^*_{21} \qquad \text{for all } C_1,C_2,\psi_1,\psi_2 \quad (* = \text{tracer})$$

and that tracer equilibrium is independent of the distribution of the abundant isotope, so that:

$$K_{12}/K_{21} = K^*_{12}/K^*_{21} = \exp\{\frac{ZF}{RT}\Delta\psi\} \qquad \text{for all } C_1,C_2,\psi_1,\psi_2$$

Therefore: $$\frac{J_{12}}{J_{21}} = (K_{12}C_1)/(K_{21}C_2) = (C_1/C_2)\exp\{\frac{ZF}{RT}\Delta\psi\}$$

W-PM-M3 DEPENDENCY OF RATE OF BIOLOGICAL ELECTRON TRANSPORT ON PROTEIN MOTION AND AVAILABILITY OF PHONON LEVELS. Brian J. Hales, Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

The temperature dependency of the rate of electron transport in biological systems was first investigated by DeVault and Chance (Biophys. J., 6, 825 (1966)). Several researchers now feel that this temperature dependency can best be explained in terms of a quantum mechanical tunneling. Data has been obtained in our laboratory on the temperature dependency of the electron transport from Fe(II) Cyt c_2 to the oxidized primary donor unit in the photosynthetic bacterium, Rhodospirillum rubrum. Comparison of this data with recent temperature dependency data obtained on similar systems shows a reoccurring inflection in the rate of transport in the temperature range 100-130°K. This inflection is independent of the system investigated and can be assigned as an inherent property of proteins. A model system will be discussed which shows that side-chain motion in proteins influence their phonon levels. The temperature dependency of this motion correspondingly effects the rate of electron tunneling and yields the protein's characterization inflection mentioned above.

W-PM-M4 A SIMPLE APPROXIMATION FOR THE CALCULATION OF THERMODYNAMIC PROPERTIES OF IONIC SYSTEMS. Wilmer Olivares and Donald A. McQuarrie, Department of Chemistry, Indiana University, Bloomington, Indiana.

We present a simple but numerically accurate approximation scheme, suggested by recent developments in the statistical mechanical theories of ionic solutions, to calculate thermodynamic properties of ionic systems. Although these theories themselves yield exceedingly good comparison with Monte Carlo calculations, they involve fairly advanced theoretical and mathematical techniques and do not appear to be readily solvable for other than very simple geometries. The approximation suggested here requires only the solution of the linear Debye-Hückel equation, an equation which has been solved for a variety of systems. The essence of this approximation is first to formulate the thermodynamic properties in terms of the interionic radial distribution function $g_{ij}(r)$, to write this as

$$g_{ij}(r) = g_{ij}^{HS}(r) \exp(-\psi_{ij}^{DH}(r)/kT)$$

where $\psi_{ij}^{DH}(r)$ is the Debye-Hückel potential and $g_{ij}^{HS}(r)$ is the well-known hard sphere radial distribution function, and to parametrize $\psi_{ij}^{DH}(r)$ by requiring that electroneutrality be satisfied.

When applied to the restricted primitive model of 1-1 and 2-2 electrolytes, this scheme yields results that are in very good agreement with the available Monte Carlo data. Preliminary calculations on the double layer problem and on the prediction of protein titration curves substantiate the applicability of this technique.

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W-PM-M5 ON THE APPLICABILITY OF CONTINUUM REACTION FIELD THEORY FOR PURE LIQUIDS AND SOLUTIONS. S. Nir, Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14203.

The coupling of free-space quantum mechanical calculations with continuum reaction field treatment of solvent effects may be a promising way of predicting conformations of macromolecules in various solvents. Two contributions to the free energy of interaction of a molecule with the entire liquid environment are discussed, (a) the interaction of a polarizable dipole with the entire polarizing medium, and (b) dispersion interactions. The expressions for the free energy of interaction include many body effects and thermodynamic fluctuations. Linder (1) obtained reasonable agreement with experimental data for the heat of vaporization of several simple liquids. Following the approach of Linder (2) similar agreement with experimental results was obtained for the case of other liquids, including water, when the polarizabilities were determined from a dispersion equation which included infrared data. No adjustable parameters are used in calculations of the components (a) and (b) discussed. Introduction of various acceptable functions for repulsive interactions into free energy calculations, further improve agreement with experimental data for the heat of vaporization. Introduction of another parameter, the free volume, enables prediction of vapor pressures at various temperatures to be made.

(1). B. Linder, J. Chem. Phys. 33, 668 (1960), 35, 37 (1961).
(2). B. Linder, Adv. Chem. Phys. 12, 225 (1967).

W-PM-M6 EVOLUTION OF THE CELL POTENTIAL AS AN ENERGY CONSERVING MECHANISM.

David F. Hastings, Duke University Marine Laboratory, Beaufort, North Carolina, 28516.

Most biological cells have a negative cytoplasmic potential. Early selection pressures favored the initial evolution of high K^+ cells with negative potentials. After more than a billion years, all the living kingdoms have retained this characteristic, an unbeatable record for conservative evolution; a record which could only be achieved by continued selection pressure. What are the modern selection pressures? I suggest that evolving cells adjust their ionic composition and membrane properties to minimize the energy expended on growth and the power expended on maintaining the steady state. The variables in the energy and power functions are the permeability properties of the membrane and the concentration of the permeant ions. Given the ionic composition of the environment and the net valence of the impermeant cytoplasmic components, one can solve the energy functions for conditions of minimum energy expenditure. For bathing media with high Na/K ratios and for cytoplasm with a negative colloidal charge the minimum occurs at a negative membrane potential and high cytoplasmic K^+ concentration. The observed high Na/K ratio in surface waters reflects the relative abundance and solubility of these salts in the earth's crust. The negative colloidal charge has evolved with the cell; carbon metabolism utilizes carbonic acids and phosphorylated intermediates, replication utilizes nucleic acids, and energy manipulation utilizes phosphate polymers. The anionic acids greatly outnumber the cationic amines which occur primarily as zwitterions in the amino acids. Thus the selection pressure for a negative membrane potential is linked to the geology of the earth's crust and the chemistry of life. (Supported by NIH Grant #3P01-HL12157.)

W-PM-M7 FORCE BALANCES IN POLYELECTROLYTE SYSTEMS. Bruce E. Enos and Donald A. McQuarrie, Department of Chemistry, Indiana University, Bloomington, Indiana 47401.

Equilibrium separation distances in a model system composed of two spherical polyions is analyzed as a function of the pH and ionic strength of the bathing solution. The separations are determined graphically as a force balance between electrostatic repulsion and van der Waals attraction. From basic input such as the surface density of ionizable groups and the corresponding dissociation characteristics of these groups, a self consistant functional of the potential is developed from which the electrostatic repulsive force may be obtained. The van der Waals attractive force is obtained from the Hamaker functional form for the interaction energy between spheres, with a Hamaker constant chosen by fitting the asymptotic form of the Hamaker expression to a more rigorous form based on the Lifschitz theory. Application of this theory is made to a simplified model of the movement of synaptic vesicles in neurons. Allowing the radius of one sphere to become very large, the resulting sphere-plane system is used to model the interaction of a synaptic vesicle with the presynaptic membrane.

W-PM-M8 IS THE PHOTON IN PHOTOSYNTHESIS HEAT OR WORK? David B. Shear, Department of Biochemistry, University of Missouri, Columbia, Mo. 65201.

The chloroplast is a biological heat engine which absorbs quanta $h\nu$ of energy from the hot solar radiation field at (effective) temperature $T_R(\nu)$. Some fraction of this energy shows up as heat flow q' to the surroundings at ambient temperature $T<T_R$. The chloroplast is an *open, steady state* chemical machine mediating biosynthesis, the net effect of which appears entirely in the surroundings. If the prime refers to the surroundings and R denotes the radiation field,

$$dE' = dE_{chem} + q' \qquad dS' = dS_{chem} + q'/T$$
$$dE_R = -nh\nu \qquad dS_R = -nh\nu/T_R$$

where all changes are per unit time. The first law of thermodynamics says that $dE'+dE_R=0$, while the second law requires that $dS'+dS_R>0$. The result is that

$$nh\nu(1-T/T_R) > dE_{chem} - TdS_{chem} = dF'.$$

Thus dF', the Helmholtz free energy gained by the surroundings, has as an upper limit the absorbed light times the Carnot factor $0<(1-T/T_R)<1$. Looking inside the "green box" chloroplast which attempts to make optimum use of the radiant flux falling upon it, there is a compromise between thermodynamic efficiency *per photon* and the desirability of processing the maximum amount of incident light. The *operational* Carnot factor, $(1-T/T_e)$ with $T_e<T_R$, will depend on the ratio of electronically excited to ground state chlorophyll molecules.

W-PM-M9 AN ANALYSIS OF HYSTERESIS IN THE POLY-GLUTAMIC ACID-Ca^{++} MEMBRANE. L. Y. Huang, Physiology and Biophysics Dept., Mayo Clinic, Rochester, Minnesota, and R. A. Spangler, Dept. of Biophysical Sciences, SUNY at Buffalo, Buffalo, N.Y.

Several years ago, Shashoua demonstrated oscillation in unsymmetric polyelectrolyte membranes. In further examination of the electrical properties of the polyglutamic - Ca^{++} membrane, we found a region of distinct hysteresis in the voltage current curve for this system. A sharp transition from a state of low membrane resistance to one of high resistance occurs at a current density different from that of the inverse transition.

This membrane system has been modeled as a two layer structure: a negatively charged layer of ionized polyelectrolyte, in series with a neutral region in which the polymeric ionic sites are masked by calcium ion. This idealized structure presents a discontinuity in the mobile ion (KCℓ) transference numbers resulting in accumulation (depletion) of salt at the interfacial region during current flow. Changes in membrane polymeric conformation, induced by the altered salt concentration, in turn affect the membrane conductance and the rate of salt accumulation. Based upon non-equilibrium thermodynamic flow equations, and a two-state representation of conformal change, our model displays a region of hysteresis and provides a plausible interpretation of the membrane phenomenon observed.

W-PM-M10 PRESCRIPTIVE RELATIONAL BIOLOGY.
O.E. Rössler†, Division of Theoretical Chemistry, University of Tübingen, 74 Tübingen, W. Germany. (Intr. by C. Walter.)

Relational biology, in its deductive branch, consists in the construction of abstract biologies by means of (1) a postulated constitutive function, defining a whole equivalence class of systems, and (2) a postulated set of optimality constraints, effectively restricting the class. It thus formalizes teleonomy. The most successful hypothetic constitutive function is the Lamarck-Darwinian one (self-maintenance at the population level in a distinct environment). A drawback is its intrinsic iterative nature (letting history enter through the backdoor of the constraints), such that at best 'explanations', but not 'predictions' or even 'prescriptions', become possible. There is one exception, however, (allowing for ordinary simplicity constraints, and prescriptive results on their basis): environments showing spatio-temporal regularities in the distribution of metabolically significant factors. They have to be exploited by purely spatio-temporal means (so that history is cut off in effect). Thus, locomotion in gradients and in point-distributions, respectively, must obey certain prescribed rules (putting biology to test). Judging from data taken from recent experimental literature, bacterial locomotion in smooth gradients closely matches the abstract equation. Further experiments (e.g., on the existence in the bacterial 'brain' of two distinct centers of computation) can be suggested. 'Higher' functional brains (coping with more complicated spatial environments) can be derived and verified also.

W-PM-M11 CONFORMATIONAL MODEL OF MEMORY AND MEMORY MANIPULATION. Michael Conrad, Department of Biology, The City College of the City University of New York, New York, N.Y. 10031.

There is compelling evidence that the memory system of the brain does not suffer serious problems of memory superposition. This is possible if: 1) certain neurons (called primaries) firing in response to external inputs undergo sensitization at the dendrites; 2) the sensitized primaries are modified by other (reference) neurons and in such a way that they fire in response to the future firing of these reference neurons. The reference neurons are either activated in temporal order, allowing for a time ordered memory structure, or are modified and later activated by primaries, thus allowing for either content ordered or associative memory structures. The amount of neural tissue required is considerably reduced if the system utilizes party line organization, in which case the communication among the neurons must be mediated by neural codes and the modification and calling processes by conformational changes at the molecular level. The model allows for rememorization on the same basis as memory acquisition and retrieval, and therefore for the stability of the memory trace (through duplication of molecular conformations) and general powers of memory manipulation (through recombination of memories). It also allows for computation with global representations of the environment; makes predictions about the biochemical correlates of learning and memory, the structure of the cerebral cortex, the distributed and localized aspects of the memory trace; and accounts for the details of classical conditioning and instrumental learning.

W-PM-M12 USE OF TIME DEPENDENT POLYNOMIAL EXPRESSIONS FOR FOLLOWING BIOLOGICAL GROWTH AND IN COMPARING POPULATIONS. Richard P. Spencer and Burton J. Lang.* Department of Nuclear Medicine, University of Connecticut Health Center, Farmington, Connecticut 06032.

The control mechanisms for many biological growth processes are unknown. Hence empirical expressions have been used to describe such measurables as weight or volume. Some give the time-dependent volume change as a function of volume itself. In others, it is entirely time dependent. One of the simplest descriptions would be $dV/dt = bt + c$. There are several reports in which the integrated form of the equation (polynomial) gives a close description of growth events such as that of the rat hepatoma 3924A and of an induced skin tumor in mice. We have used the polynomials in 3 interesting ways. I. To determine the effects of therapy on growth by comparing pre- and post-treatment curves. II. To estimate the age of wild animals (in their developmental stage) by use of body weight. Based on the data of the Morris', an excellent fit can be obtained for the weight of the giant panda over the first 16 months of life, by forcing the curve through the known birth weight. III. To compare populations in more complex growth situations (in these cases, polynomials of an advanced order may have to be used). For example, the width of the human metacarpal bone peaks at about age 10 years, decreases to a minimum near age 35, and finally increases thereafter. To fit this curve, a fourth order polynomial can be used (and close agreement is obtained). While this does not give insights as to controlling factors, it does allow a comparison of males and females, and between populations from different parts of the world. (Supported by USPHS CA 14969 and by DT-34E from the American Cancer Society).

W-PM-M13 PHYSICAL THEORY OF GENETIC STABILITY. W.G. Cooper* and J.F. Pierce* (Intro. by H.B. Gray,Jr), Department of Physics, M.D. Anderson Hospital & Tumor Institute of The University of Texas System Cancer Center, Houston, Texas 77025 and Physics Department, University of Houston, Houston, Texas 77002.

A dynamic model of in vivo DNA is given which predicts that (a) uninduced "point" mutations can arise as a consequence of microscopic properties associated with intermolecular hydrogen bonds and that (b) classical motion of macroscopic DNA can alter lesion rates. The model is constructed to satisfy temperature dependent mutation data of metabolizing and nonmetabolizing T-4 viruses. It is illustrated by discussing microscopic lesions at the G-C base pair which occurred in the absence of metabolism, but manifested as transversion and transition mutations at replication. Rearrangement of two H-bonded protons at the G-C base pairs accounts for the genetic data and is compatible with chemical stability requirements. This intermolecular proton exchange is treated as a two particle system in a four well potential using time dependent perturbation theory and a two state process. Order of magnitude calculations indicate compability of energy barrier dimensions with reaction rates. Motion of the macroscopic system is in terms of torsional and longitudinal oscillations which form nodes and antinodes along a cylindrical axis of DNA. Standing wave resonance interference allows the "breathing" phenomena to occur at nodes, but requires a compression at antinodes. The microscopic transition rates increase at such antinodes which is consistent with "hot spot" mutations. The model suggests new types of experiments and is susceptible to further refinements. Supported in part by Public Health Fellowship GM-57206-01 and NIH Grant CA 5099.