F-AM-A1 A MATHEMATICAL MODEL FOR NON-ISOVOLUMIC SHORTENING IN SKINNED STRIATED MUSCLE FIBERS. E. W. April and D. Wong*. Department of Anatomy, College of Physicians & Surgeons of Columbia University, New York City, New York 10032.

The lattice of thick filaments in striated muscle can be considered liquid-crystalline in nature. It has been shown that the lattice state may be one of either electrical balance between van der Waal's and electrostatic forces or one of volume constraint due to the active and passive properties of the sarcolemma (E. W. April, Nature 257:139-142, 1975). The unit-cell volume of the filament lattice of skinned muscle fibers does not behave in a constant volume manner with change in sarcomere length. This particular behavior can be explained in terms of the net negative charge within the A-band. Not only does the electrostatic force between the filaments change with alteration in ionic strength and pH which respectively screen and alter the net charge, but also with amount of filament overlap. Thus, total A-band charge at any given value of pH and ionic strength is a function of the sarcomere length. In the absence of a volume constraint, i.e., the sarcolemma, the thick filaments reposition to maintain a state of minimum interaction energy. By determining the effective charge density on the thick and thin filaments, it can be demonstrated that net charge in the A-band unit cell ($Q_A$) is a function of filament overlap. Since interfilament distance (d) is also a function of sarcomere length, it can be shown that $d \propto Q_A$. This mathematical analysis is consistent with the experimental data and is supportive of the concept that electrostatic repulsive forces maintain the smectic liquid-crystalline structure of the A-band of striated muscle. (Supported by grants from USPHS and MDAA)

F-AM-A2 A CRUSTACEAN MUSCLE WITH UNUSUAL THICK AND THIN FILAMENT PACKING. A. B. Eastwood*, D. S. Wood*, and J. P. Reuben. Dept. of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

The posterio-ventral portion of the proximal accessory flexor in the meropodite of crayfish (Orconectes sp.) is composed of small diameter (2-10 μm) fibers. These fibers develop maximum tension (1-2 Kg/cm$^2$) only when stretched approximately 2 x slack length ($L_s$). At $L_s$, tansions induced by 100-200 mM K or 20 mM caffeine are 10-20% of maximum. After fixation at or near $L_s$, electronmicroscopic examination reveals that at the center of the A band, myofilaments are segregated into regions containing almost exclusively thick or thin filaments. Away from the A-band center, thick filaments are surrounded by orbits of 12 thin filaments and regions of exclusively thin filaments persist. After fixation at 2 $L_s$, a normal crustacean pattern with orbits of 12 thin filaments surrounding each thick filament and no exclusively thin filament region is observed throughout the A-band. The length-active tension curve is skewed with a long linear rise below 2 $L_s$ and a sharp fall off beyond 2 $L_s$. The muscle in vivo works exclusively in the linear, rising portion, and this may be related to the function of the myochordotonal receptors. The phenomena involved in producing the unique thick-thin filament distribution at $L_s$ remain to be determined. Supported in part by H. Houston Merritt Clincial Research Center for Muscular Dystrophy and Related Diseases and the NINDS.

F-AM-A3 SARCOMERE STRUCTURE AND FUNCTION FOLLOWING EXTREME SHORTENING OF FROG SKELETAL MUSCLES. R.J.M. McCarter* (Intr. by T.C. Smith), Physiology Dept., University of Texas Health Science Center, San Antonio, Texas 78284

The ultrastructure and the movement of sarcomeres under external isometric conditions were determined for frog sartorius muscles at 21°C, using the techniques of electron microscopy and optical diffraction. Extreme shortening was induced in a series of four 2S tetani given at 10-minute intervals. The final contracted length of the muscles corresponded to average sarcomere spacings of less than 1 μm. Sarcomeres of muscles fixed at this length showed 3 characteristic forms: (a) major lateral disorder of thick filaments and Z discs, folding of thick filaments against Z discs; (b) lateral order of thick filaments and Z discs maintained, some folding of thick filaments against Z discs; (c) intact lateral order with ends of thick filaments close to Z discs. When the muscles were stretched back to the original resting length (RL, length of muscle in vivo) and to 120%RL and 130%RL, two characteristic patterns emerged: (a) sarcomeres in which only the ends of the thick filaments were distorted (i.e., no sharp edges for A-band, but distinct edges for H-zone); (b) sarcomeres with major lateral displacement of thick filaments, such that no marked banding (Other than due to Z discs) was present. The active isometric length-tension diagram obtained following extreme shortening showed surprisingly little variation from muscle to muscle, and exhibited a maximum active tension of 43±1% (mean ± S.E.M., n=8) of the normal value. Short exposure (1.5 mS) laser diffraction patterns obtained during isometric tetanic contractions at extended lengths (≥ RL) suggest movement about a mean value rather than net changes in sarcomere spacing during contraction. Well defined diffraction patterns were obtained from the resting muscles despite the major lateral disorganization of thick filaments present.

F-AM-A4 ANALYSIS OF PHOSPHATE METABOLITES, THE INTRACELLULAR PH, AND THE STATE OF ATP IN INTACT MUSCLE BY $^{31}P$ NMR. C. Tyler Burt, T. Glonek*, and M. Bárány, Biological Chemistry Department and Research Resources Center, University of Illinois at the Medical Center, Chicago, Illinois 60612

$^{31}P$ NMR spectra recorded from intact muscles showed resonances for ATP, P-Creatine, $P_i$, and the sugar phosphates. Quantitation of these metabolites by $^{31}P$ NMR was in good agreement with values obtained by chemical analyses. The spectra obtained from various muscles showed considerable variation in their phosphorus profile. Thus differences could be detected between: 1) normal and diseased muscle; 2) vertebrates and invertebrates; 3) different species of the same animal. The time course of change in phosphate metabolites in frog muscle showed that the ATP level remains unchanged until P-Creatine is nearly depleted.

Several lines of evidence indicated that ATP is complexed with Mg in the muscle water: 1) the phosphate resonances of ATP in the muscle were shifted downfield as compared to those in the alkaline earth metal free perchloric acid extract of the muscle; 2) the coupling constants of ATP measured in various live muscles closely corresponded to those for MgATP in a solution resembling the composition of the muscle water; 3) in the muscle the $\gamma$-phosphate group of ATP exhibited no shift-change over a period of 10 hours under conditions where resonances of other phosphate compounds could be titrated. This behavior is similar to that of MgATP in model solutions in the physiological pH range, and it is different from that of CaATP.

The chemical shifts of the phosphate metabolites were determined in several relevant solutions as a function of pH. From the chemical shift of $P_i$ observed during aging of intact muscle the intracellular pH of frog muscle was estimated to be 7.2. (Supported by MDA, MDAC, and NS 12172 from NIH).

F-AM-A5 EFFECT OF INORGANIC PHOSPHATE ($P_i$), SUBSTRATE (MgATP) AND EXCESS ATP ON COMPLEX STIFFNESS OF SKINNED CRAYFISH MUSCLE FIBERS AND GLYCERINATED RABBIT PSOAS MUSCLE BUNDLES. M. Kawai*, and M. Orentlicher, Dept. of Neurology, College of Physicians & Surgeons, Columbia University, New York, N. Y. 10032

Three exponential processes can be resolved by analysis of tension responses to sinusoidal length changes of intact crayfish muscle fibers. They are (a) slow lead (rate constant: 6/sec., 20° C); (b) lag at middle frequency (140/sec., oscillatory work), and (c) fast lead (600/sec). These three time courses are present in fully activated skinned crayfish or glycerinated psoas preparations, but with these preparations, their relative magnitudes depend largely on $P_i$ and substrate concentration, and not on ATP. Oscillatory work is totally missing in standard activating conditions ($Ca^{++} > 10$ μM, $MgATP^{=} = 1$ mM), and it first appears at 5 mM of substrate. The oscillatory work is greatly enhanced by the addition of 2-4 mM of $P_i$ to the bathing medium. As White and Thorson (1972) found, addition of $P_i$ increases the optimum frequency of the oscillatory work, but in contrast to their finding $P_i$ also enhances the magnitude of the oscillatory work loop on Nyquist plot dramatically. Increasing the substrate concentration up to 20 mM has a similar but smaller effect. The magnitude of higher frequency lead is relatively unchanged by $P_i$ or substrate but the associated rate constant (c) is increased by these treatments. Altering the ATP concentration from 1-15 mM above the substrate concentration has little effect on the above parameters when magnitudes are scaled by tension. Supported in part by H. Houston Merritt Clin. Res. Ct. for Muscular Dystrophy & Related Diseases and NINDS.

F-AM-A6 EFFECTS OF TETRACAINE ON CONTRACTION AND "GATING CURRENTS" IN FROG SKELETAL MUSCLE. W. Almers and P.M. Best*, Dept. Physiology, Univ. Wash. Sch. Med., Seattle, Wash. 98195

The local anesthetic tetracaine (Tet) can block contraction of intact muscle at submillimolar concentration. However, when the sarcolemma is mechanically removed from a frog semitendinosus fiber and $Ca^{++}$ applied directly to the contractile proteins, we find that contracture tension is undiminished by 2 mM Tet. In fact, the drug seems to sensitize such a "skinned" fiber to $Ca^{++}$, shifting the tension-$[Ca^{++}]$ relation to two times lower concentrations. Tet therefore does not incapacitate the contractile proteins and must instead act by blocking $Ca^{++}$-release from the sarcoplasmic reticulum (SR). Caffeine contractures in skinned fibers are depressed or blocked by 2 mM Tet, but not $Cl^{-}$-induced contractures.

Next the three-electrode voltage clamp technique was used to study what are thought to be "gating currents" linking $Ca^{++}$-release from the SR to cell membrane depolarization. We find that 2 mM Tet, though blocking the contractile response to depolarization, has little or no effect on size or kinetics of these dielectric "gating currents." Therefore, if the latter are involved in regulating $Ca^{++}$-release, then Tet must be able to block the physiological $Ca^{++}$-release site on the SR without interfering much with the release regulating mechanism.

One might suppose that muscle "gating currents" play a role in regulating instead the delayed $K^{+}$-channel in the sarcolemma. However, 2 mM Tet were found to strongly affect the gating of that channel, both slowing its voltage dependence and shifting it to 20-30 mV more positive potentials. Considering Tet's lack of effect on "gating currents" this suggests that the dielectric currents observed in muscle for the most part have nothing to do with the delayed $K^{+}$-channel. Tetraethylammonium ions are another agent known to affect gating of that channel by slowing its kinetics fivefold. Again, no effect of this agent (180 mM) on muscle "gating currents" was found. Supported by PHS grant no. AM17803.

**F-AM-A7** K ACCUMULATION AND K CURRENTS IN VOLTAGE CLAMPED FROG VENTRICULAR MUSCLE.
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Combining a single sucrose gap voltage-clamp technique with a K electrode, the extracellular K accumulation following long clamp pulses (1 to 8 sec) was estimated from measurements of after potential, action potential duration, and K activity and were found to yield N-shaped relations (maximum at -40 mV and minimum at -20 mV) similar to the steady state current-voltage relation. Analysis of current traces with respect to accumulation of K showed that an inward-rectifying current ($I_K$) dominates below -20 mV and a time-dependent K current activates above -20 mV. The current-voltage relation for $I_K$ has a maximum at -60 mV and a region of marked negative slope between -50 and -20 mV, when measured by linear extrapolation of the current records to the beginning of the clamp pulse. The shape for the $I_K$ current-voltage relations remains unchanged when $K_o$ is changed by addition of K or post-clamp accumulation. However, the equilibrium potential for this current changes shifting the curve along the voltage axis. The deviation between the steady state I-V relation and the $I_K$ currents below -20 mV may be entirely accounted for by K accumulation. Current records and accumulation obtained above -20 mV indicate that time-dependent K currents are insensitive to changes in $K_o$. K-enhanced currents result only from the shift of $I_K$ to less negative potentials, effectively increasing the net membrane current in the negative conductance region. These results suggest that shortening of the action potential in the presence of increased $K_o$ is due to currents activating around -60 to -20 mV rather than those activating at the plateau (+30 to 0 mV) and are consistent with the K electrode measurement indicating no change in K efflux during the plateau on alteration of $K_o$. Supported by USPHS HL 16152.

**F-AM-A8** OPTICAL MEASUREMENTS OF CARDIAC ACTION POTENTIAL WITH MEROCYANINE 540.
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Fluorescence and absorption measurements from frog hearts stained with Merocyanine 540 were used to monitor the action potential. When bound to the myocardium, Merocyanine 540 has a major absorption peak at 540 nm and a minor peak at 570 nm. With either a 540 or a 570 nm excitation wave length, the fluorescence peaks at 585 to 590 nm. During the plateau, the fluorescence intensity increased by 1.5 to 2.0% when excited with a 540 nm light. With one mm excitation beam focused on various regions of the heart, pacemaker, atrial, and ventricular action potential could be recorded. The signal to noise ratio from a single action potential ranged between 10/1 to 40/1. Coincident with the potential dependent fluorescence enhancement, the absorption decreases at 540 nm and increases at 570 nm. Although the absorption measurements are of opposite directions, their time course is similar to the fluorescence action potential. The signal to noise ratio of absorption action potential at 540 nm is 10 times larger than at 570 nm. Rapid scan spectroscopy ($\backsim$20 msec) with silicon-diode Videcon system showed that the fluorescence emission increases in intensity with no wave length shift during the action potential. Physiological interventions (frequency, temperature) and pharmacological or ionic agents do not alter the relation between the fluorescence signal and the intracellular microelectrodes. These results suggest that Merocyanine 540 can be used to measure electrical activity of various regions of the heart without any apparent toxic effects on the heart. Supported by USPHS HL 17702.

**F-AM-A9** DYE ABSORPTION CHANGES DURING EXCITATION OF SINGLE AMPHIBIAN MUSCLE FIBERS.
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Single muscle fibers of Xenopus or frog were stained with merocyanine 540 (5$\mu$g/ml) for 10 min. and light absorption changes (Ross et al. Biophys. J. 14, 983) during excitation and contraction were recorded. With the use of a microscope (objective, X 16, N.A. 0.35), the light coming from about 0.5 mm or 1 mm length of fiber was collected. After the stimulation (massive, transverse) we observed three main components of absorption changes. The first wave started immediately after the stimulus, and reached a peak at about 1.5 ms. With 519 nm light, this wave represents an increase of light (decrease of absorption) with a magnitude of $6 \times 10^{-4}$ relative to the background. With 575 nm this wave was reversed, (i.e., increase of absorption) the magnitude being $7 \times 10^{-4}$. Thus, in analogy with the result of squid axon (Ross et al.), this wave probably represents the membrane potential changes. The second wave had a peak at 2-4 ms, the third wave was long-lasting. These two waves did not change direction according to the change in light wavelength, suggesting that they are not related to membrane potential. Thus, the second and the third wave may represent latency-relaxation and contraction. In five fibers optical changes were recorded simultaneously with membrane potential changes using intracellular electrodes. The time course of the action potential was faster (peak 0.7 ms) than that of the first wave of light absorption. This suggests that the first wave represents the action potential of the T-system and surface membrane, whereas the intracellularly recorded potential is mainly a reflection of the surface membrane. Supported by NIH Grant NS-08601 and Muscular Dystrophy Association.

F-AM-A10 AGONIST-INDUCED DISCRETE CONDUCTANCE CHANGES IN FROG MUSCLE. Erwin Neher* and Bert Sakmann* (Intr. by E. Boulpaep), Dpt. Physiol. Yale Univ. New Haven, and Max Planck Institut fuer biophysik. Chem. Goettingen, W. Germany

Spectral analysis of acetylcholine(ACH) induced endplate currents at the frog neuromuscular junction suggests that ACH opens ionic channels each with a conductance of 20-30 pmho. However, the interpretation of such analysis depends on assumptions about the time-course of the conductance change of an individual channel (e.g. Katz & Miledi, J.Physiol. 224:665, 1972; Anderson & Stevens, J.Physiol. 235:655, 1973). So far it has not been possible to resolve discrete conductance changes during channel opening and closing, due to excessive noise in the experimental system. We have employed a different method for measurement of membrane current, in which the total background noise can be reduced to 1-2 pA. Using this method, we have observed discrete steplike changes in membrane current which are present only when agonist is applied. Each current pulse appears to be a square wave: a channel rapidly reaches a steady conductance which is maintained until the channel closes. Preliminary analysis of the records indicates that the single channel conductance is about 25 pmho, while the channel mean open time is dependent on membrane potential and agonist, as expected from noise analysis. For example, using suberyldicholine at 8°C, -80 mV on denervated frog M. cutaneous pectoris fibers, the channel mean open time is 27 msec and the mean open channel conductance is 24 pmho. The method employed limits current measurement to a small patch of membrane, which is isolated electrically by means of an extracellular glass pipette.

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F-AM-A11 MECHANISM OF INHIBITION OF RAT DIAPHRAGM CHLORIDE PERMEABILITY BY AROMATIC CARBOXYLIC ACIDS. P.T. Palade*and R.L. Barchi* (Intr. by A.P. Somlyo), Departments of Biology, Biochemistry & Biophysics, and Neurology, University of Pennsylvania, School of Medicine, Philadelphia, Penna. 19174

Inhibition of chloride conductance in rat diaphragm fibers at pH 7.0 and 35° C by a series of aromatic carboxylic acids was studied using a standard two electrode three point cable analysis technique. Nearly complete inhibition of $g_{Cl}$ could be produced by these compounds without effect on resting cation conductances, and the inhibition of $g_{Cl}$ was found to be readily reversible. Concentrations producing a 50% inhibition of $g_{Cl}$ were determined. A plot of these half-maximal concentrations for each compound vs. their partition coefficients as calculated by the methods of Hansch demonstrate a linear relationship, suggesting involvement with either an intramembrane or transmembrane site of action. Observations incompatible with a surface charge mechanism of action included determinations of the zeta potentials of model systems, lack of competing action by raised $Ca^{++}$, and current-voltage relationships. Simple obstruction of permeation was similarly ruled out. Experiments involving determination of anion conductance sequences following rapid solution changes and after equilibration, as well as determination of anion permeability sequences by measured transient shifts of R.P. following rapid replacement of external chloride by test anion, were performed in the presence and absence of aromatic acids. All sequences showed Cl>Br>I in control fibers and I>Br>Cl at saturating concentrations of aromatic acids suggesting an induced inversion by these acids of the apparent membrane mobility of these ions. These results are consistent with a mechanism involving interaction between the aromatic acids and an intramembrane site which controls effective ion mobility. Models for such a mechanism are presented.

**F-AM-B1** SODIUM EFFLUX FROM THE GARFISH OLFACTORY NERVE. V. Scruggs*, (Intr. by J. Zengel). Department of Physiology and Biophysics, School of Medicine, University of Miami, Miami, Florida 33152.

Olfactory nerves from the garfish Lepisosteus platyrhincus were incubated at room temperature for 1.5-2 hours in gar ringer containing 0.2mM ouabain, 75μM veratridine, and 4-6μC/ml $^{22}Na$. Each nerve was then placed in a chamber which was perfused at a constant rate with a solution identical to the incubation medium but without $^{22}Na$. The perfusate was collected and the radioactivity present in the nerve during the perfusion was calculated and plotted versus time. From these curves the fraction of labeled sodium leaving the nerve bundle per minute ($k_{app}$±SE(n)) was measured during the 20 to 30 minute period following removal of the nerve from the loading solution.

In 3 nerves the apparent rate constant $k_{app}$=0.0643±.0003 $min^{-1}$ (3). Incubating these nerves in gar ringer similar to above but also containing 0.5μM tetrodotoxin decreased the $k_{app}$ to 0.0282±0.002 $min^{-1}$. When the experiment was repeated with 1mM Na and 124mM Tris replacing the 125mM Na in normal gar ringer, the $k_{app}$ was 0.045±0.003 $min^{-1}$(3) and addition of tetrodotoxin did not change the rate constant, $k_{app}$=0.045±0.003 (3). These preliminary results support the idea that the tetrodotoxin sensitive efflux which occurs in the presence of ouabain and veratridine is dependent on the concentration of sodium ions bathing the nerve.

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**F-AM-B2** INTRODUCTORY STUDY OF THE SODIUM PUMP IN MYXICOLA GIANT AXONS. R.F. Abercrombie,* and R.A. Sjodin, Department of Biophysics, University of Maryland School of Medicine, Baltimore, Md. 21201

Giant axons from the marine annelid Myxicola *infundibulum* have been microinjected with $^{22}Na$ to study the properties of Na efflux. The efflux of Na was observed to depend upon $[K]_o$ and $[Na]_o$. Removal of $[K]_o$ from seawater ($[K]_o$=10 mM) reduced Na efflux to 64 ± 2% of its normal value. External application of $10^{-4}$M ouabain decreased Na efflux to 35 ± 3% of its normal value and abolished sensitivity to $[K]_o$. At values of $[K]_o$ between 10 mM and 35 mM, removal of $[Na]_o$ by replacement with a Mg-mannitol mixture, mannitol alone, or neutralized Trizma base reduced Na efflux. The Na efflux that was dependent on $[Na]_o$ was eliminated by application of ouabain. Under K-free conditions, removal of $[Na]_o$ increased the Na efflux by about 30%. It is concluded that both $[K]_o$ and $[Na]_o$ activate a ouabain-sensitive Na pump in Myxicola giant axons. The actions of $[K]_o$ and $[Na]_o$ at external pump activation sites are apparently competitive as increasing $[K]_o$ to 50 mM or higher made Na efflux insensitive to $[Na]_o$. In comparison with giant axons from the squid, Myxicola giant axons show a somewhat lower sensitivity to $[K]_o$ and an ability of $[Na]_o$ to activate the pump under normal conditions. Activation by $[Na]_o$ has been observed in biochemically abnormal squid giant axons but the effects of $[Na]_o$ are variable in untreated squid axons.

**F-AM-B3** IONIZED CALCIUM CONCENTRATIONS IN SQUID AXONS. R. DiPolo, J. Requena*, F. J. Brinley, Jr., L. J. Mullins, A. Scarpa, and T. Tiffert*. Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela; Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md. 21205; Department of Biophysics, University of Maryland School of Medicine, Baltimore, Md. 21201; Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174.

Values for ionized [Ca] in squid axons were obtained by measuring the light emission from a 0.1 μl drop of aequorin confined to a plastic dialysis tube of 140 μm diameter and located at the center of a squid axon. Ionized Ca had a mean value of 20 x $10^{-9}$ M as judged by the subsequent introduction of CaEGTA/EGTA buffer into the axoplasm, and light measurement on a second aequorin drop. With a freshly isolated axon in 10 mM Ca seawater, the aequorin glow invariably increased with time; a seawater [Ca] of 2-3 mM allowed a steady state with respect to $[Ca]_i$. Replacement of $Na^+$ in seawater with choline led to a large increase in light emission from aequorin. Li seawater partially reversed this change and the reintroduction of $Na^+$ brought light levels back to their initial value. Stimulation at 60/s for 2-5 min produced an increase in aequorin glow about 1% of that represented by the known Ca influx, while treatment of an axon with CN produced a very large increase in aequorin glow but only if the external seawater contained Ca. [Aided by grants from NINDS (05846, 08336) and from NSF (GB41593)].

F-AM-B4 INHIBITION OF "PROTON PUMPING" BY CN AND DNP, AND STIMULATION BY $HCO_3^-/CO_2$. W.F. Boron* and P. De Weer. Washington University School of Medicine, St. Louis, Missouri 63110.

"Proton pumping" in squid giant axons was studied by monitoring intracellular pH ($pH_i$) changes with glass microelectrodes. We previously showed that exposure of axons to artificial sea water (ASW) containing 5% $CO_2$/50 mM $HCO_3^-$ causes $pH_i$ to fall abruptly, and then to recover slowly despite the continued presence of $CO_2$. This secondary recovery (which occurs against the electrochemical gradients for $H^+$, $OH^-$, and $HCO_3^-$), and the subsequent overshoot of $pH_i$ beyond its initial value when the axon is returned to normal ASW, have been ascribed to a proton-extruding pump. In the present study, axons were exposed to nominally 2 mM CN, a treatment which causes $pH_i$ to fall slowly over the course of 1-2 hrs. Once a steady $pH_i$ was achieved, these axons were exposed to a pulse of 5% $CO_2$/50 mM $HCO_3^-$ in the continued presence of CN. Although $pH_i$ fell sharply as expected, no recovery alkalinizations or overshoots were observed. After return to CN-free ASW, axons were again capable of secondary alkalinizations and overshoots in response to $CO_2$ pulses. Similar results were obtained when axons were poisoned with DNP. We also examined the effects of $HCO_3^-/CO_2$ on "proton pumping". Acid loads were effectively placed on axons by exposing them for 30 min to 50 mM $NH_4Cl$-ASW. After return to $NH_4$-free, HEPES-buffered ASW, $pH_i$ falls to values (7.0) appreciably lower than initial $pH_i$ (7.35). At an external pH ($pH_o$) of 7.4, $pH_i$ will remain at this low level unless the bathing seawater contains $CO_2/HCO_3^-$ (0.4% $CO_2$/ 2.5 mM $HCO_3^-$), in which case $pH_i$ rises rapidly (against an electrochemical gradient) after a small acidification due to the influx of $CO_2$. Stimulation of secondary alkalinization by $HCO_3^-/CO_2$ was observed at all $pH_o$ values between 6.0 and 9.0.

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F-AM-B5 ATP-DEPENDENT CHLORIDE INFLUX INTO INTERNALLY DIALYZED SQUID GIANT AXONS. John M. Russell, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Measurements were made of 36-Cl influx into axons whose internal solutes were controlled by means of internal dialysis. The influence of ATP on this influx was tested by removing ATP from the internal dialysis fluid and preventing mitochondrial ATP synthesis by including 2 mM cyanide. When the intracellular chloride concentration was 50 mM and 5 mM ATP was included in the dialysis fluid, the average influx at 12°C was 11.6 pmoles/$cm^2$·sec (n=13). When the axons were dialyzed with the ATP-free, cyanide-containing solution, the average influx fell to 5.1 pmoles/$cm^2$·sec. The effect was fully reversible upon return of the ATP to the dialysis fluid, even when cyanide was still present in the fluid. The effects of changing the intracellular chloride concentration were also studied in the presence and absence of ATP. Raising the intracellular chloride concentration to 150 mM caused the total influx to fall to an average of 3.4 pmoles/$cm^2$·sec and the ATP-independent influx to fall to 1.5 pmoles/$cm^2$·sec. Conversely, lowering intracellular chloride concentration to 14 mM caused the total 36-Cl influx to increase to 14.8 pmoles/$cm^2$·sec while the ATP-independent flux increased to 5.6 pmoles/$cm^2$·sec. (Supported by NIH grant, NS-11946).

F-AM-B6 CHLORIDE FLUXES IN THE DIALYZED BARNACLE MUSCLE FIBER AND THE EFFECT OF SITS. John M. Russell and M.S. Brodwick* (Intr. by R.D. Baker), Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

36-chloride efflux from dialyzed barnacle muscle fibers was found to have a rather high $Q_{10}$, between 3 and 4, a fact earlier reported by DiPolo (J.G.P. 60:471, 1972). DiPolo also reported that substituting $NO_3$ or methanesulfonate for external Cl caused chloride efflux to fall approximately 50%. We have confirmed this result at 20° and 25°C. However, at 15°C such trans-side inhibition can only be observed when $[Cl]_i$ is below 25-30 mM, whereas at 20-25°C it is observable with $[Cl]_i$ as high as 180 mM. Furthermore, we have observed that replacing external Cl with propionate or bicarbonate (keeping pH constant) stimulates Cl efflux. These data have suggested the existence of a membrane carrier mechanism for Cl in the barnacle sarcolemma. We tested this hypothesis by applying SITS (4-acetamido-4'-isthiocyano-2,2'-disulfonic stilbene), an agent which is known to block anion transport in erythrocytes. We found this agent to block about 50% of Cl efflux irreversibly at 20°C. In the presence of SITS, methanesulfonate caused no further inhibition and propionate did not stimulate 36-Cl efflux. Finally, after SITS application, the $Q_{10}$ for Cl efflux is decreased by one-half. These data support the hypothesis of a Cl carrier in the barnacle muscle membrane. (Supported by NIH grant NS-11946).

**F-AM-B7** TITRATABLE SITES OF $Cl^-$ CHANNELS OF FROG MUSCLE ARE NEAR THE OUTSIDE OF THE MEMBRANE. M.G. Parker* and J.W. Woodbury, Department of Physiology, University of Utah, Salt Lake City, Utah 84132

Hutter and Warner showed (1967)that the $Cl^-$ conductance ($g_{Cl}$) of frog sartorius muscle fibers depends on pH. The relationship of $g_{Cl}$ to pH is a titration curve with a pK of about 7. The effect of pH on $g_{Cl}$ is thought to be mediated by a site in $Cl^-$ channels (possibly an imidazole group) that can bind an $H^+$ ion; $g_{Cl}$ is low ($\sim$ o) when the site is protonated. The fractional distance of the $H^+$ binding site through the transmembrane potential ($E_m$) can be estimated by measuring the effect of $E_m$ on pK. The calculation is made by assuming that a potential change of 2.3RT/F = 58mv at the site changes the pK by one unit (depolarization increases pK).

Membrane conductance ($g_m$) was measured using two intracellular electrodes, a sinusoidal current (0.2Hz, 10nA) was passed through one electrode and the resulting change in $E_m$ measured with the other. The pK was estimated from measurements of $g_m$ at pH's of 5,7 and 9. $E_m$'s ranging from -10 to -90mv were obtained by altering $[K^+]$ of the perfusate. Temperature was held constant at 22 ± 0.2°C because of the high temperature dependence of the pK (Stephenson and Woodbury, these abstracts). Nevertheless, the measured pK's vary markedly from cell to cell (range 6-8). The scatter plot of pK vs. $E_m$ has the regression line pK = 7.18 + 0.0028 $E_m$ ($E_m$ < 0). The slope is not significantly different from zero (P = 0.13). (95% confidence limits of the slope are 0.0080 and -0.0024). The most probable fractional distance of the site through the membrane voltage is thus 0.0028 x 58 = 0.16 but the actual value could be anywhere between 0 and 0.5. These data indicate that the titratable sites of $Cl^-$ channels are near the outside of the membrane. (Supported by NIH Grant HL16348)

**F-AM-B8** $Cl^-$ CHANNELS OF FROG MUSCLE: CONDUCTANCE AND BINDING CONSTANT VARY SIMILARLY WITH TEMPERATURE. R.A. Stephenson* and J.W. Woodbury (Intro. by F.G. Moody) Department of Physiology, University of Utah, Salt Lake City, Utah 84132

The $Cl^-$ conductance of a frog sartorius muscle fiber increases with pH along a titration curve having a pK of about 7. The enthalpy ($\Delta H_K$) of the reaction $HS \rightleftarrows H^+ + S$ (S = binding site); $K = [H^+] \cdot [S]/[HS] = K_o \exp(\Delta H_{K/RT})$ can be estimated by measuring the temperature dependence of pK. Enthalpy of activation for $Cl^-$ permeation ($\Delta H_G$) can be estimated in the same way. Membrane conductance of frog sartorius muscle fibers was measured by passing a sinusoidal current (0.2Hz, 10nA) through one of two closely spaced intracellular electrodes and measuring the resultant change in transmembrane potential with the other. Temperature was controlled to ± 0.2°C because of the large observed effects. 5mM $BaCl_2$ was added to Ringer's to reduce $g_K$. $g_m$ of a cell was measured at 6 pH's and at 2 or more temperatures. Complete runs at 2 or more temperatures were obtained from 4 cells in 3 muscles. ΔH values in Kcal/mole are:

| | Cell 1 | Cell 2 | Cell 3 | Cell 4 | |
|---|---|---|---|---|---|
| $\Delta H_K$ | -20 | -37 | -56 | -90 | Kcal/mole |
| $\Delta H_G$ | -17 | -17 | -30 | -40 | Kcal/mole |

Individual values vary considerably but can be grouped into low (cells 1&2) and high (cells 3&4) ΔH values. There is a highly significant correlation between $\ln(g_{Cl})$ and pK; $\Delta H_K \simeq 1.5\Delta H_G$. If the widely differing ΔH's are attributable to differences in channel structure near the site, then the correlation between $\Delta H_K$ and $\Delta H_G$ values indicates that the sites controlling these processes are close together. Hutter and Warner (1967) concluded that the titratable site in $Cl^-$ channels is probably an imidazole group. The $\Delta H_K$ of the imidazole group of histidine is +8Kcal/mole, a value much different from the $\Delta H_K$ values given above. (Supported by NIH grant HL16348.)

**F-AM-B9** CHLORIDE CONDUCTANCE AND pH IN XENOPUS MUSCLE. P. Vaughan*, J. McLarnon* and D. Loo* (Intr. by R.S. Eisenberg), Dept. of Physiology, University of British Columbia, Vancouver, Canada, V6T 1W5.

The chloride conductance of sartorius muscle fibres of *Xenopus laevis* has been studied as a function of ambient pH using a three microelectrode voltage clamp. The instantaneous and steady state current-voltage relations are similar in form to those observed by Warner (J. Physiol. 227, 291-312 (1972)) for *Rana temporaria*. In order to reduce errors in the calibration of the current axes, $R_i$ has been measured, at each pH, using two-electrode cable theory techniques and assuming a fibre diameter of 70 μm. $R_i$ at pH 7.3 is 161±17 (SEM) Ω.cm. but is higher at higher pH and lower at lower pH. This has the effect of knowing the cross-over points of the steady state current-voltage relations at different pH's closer to the holding potential than is predicted assuming constant $R_i$. At pH > 8 the initial current decays to steady state with 2 time-constants: one of near 100 msec, another near 0.5 sec. This slow relaxation has a similar timecourse to the growth of current to the steady state at pH < 6. The possibility that the slow time constant is due to diffusional delays is being investigated.
(Supported by Muscular Dystrophy Association of Canada and Medical Research Council of Canada.)

F-AM-C1 BIOCHEMICAL MODEL OF SPILLOVER. E. L. Gross and D. J. Davis, Dept. of Biochemistry, Ohio State University, Columbus, Ohio. 43210

We have shown that the cation-dependent stimulation of spillover in green plant photosynthesis is due to an increased interaction between the chl a-b light harvesting pigment protein (LHPP) (Kung, S.D., and Thornber, J.P., Biochim. Biophys. Acta 253, 285 (1971). and the P700-chla protein of Photosystem I (Shiozawa, J.A. Alberte R.S., and Thornber, J.P., Arch. Biochem. Biophys. 165, 388 (1974). Two pieces of evidence support this. (1) When the P700 chlorophyll a protein is contaminated with LHPP, cations stimulate the transfer of excitation energy from chl b to the reaction centers as measured by an increase in the relative quantum yield for diphenylcarbazone disporportionation (2) Cations cause an association between the LHPP and the purified P700-chl a protein. The reconstructed system shows the same type of cation regulation of energy transfer as whole chloroplasts.

F-AM-C2 PHOTOELECTRON QUANTUM YIELDS AND PHOTOELECTRON MICROSCOPY OF CHLOROPHYLL AND CHLOROPHYLLIN. R.J. Dam*, K.F. Kongslie* and O.H. Griffith, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 97403.

The absolute photoelectron quantum yield curves of chlorophylls a and b, chlorophyllin, and phytol were examined over the wavelength range 500 nm - 180 nm. In the long wavelength region (500-240 nm) quantum yields are below $5 \times 10^{-6}$ electrons per incident photon. Below 240 nm the quantum yields rise sharply. The chlorophylls and chlorophyllin exhibit similar yield curves: measured yields were of order $5 \times 10^{-6}$ electrons per incident photon at 240 nm, $1 \times 10^{-4}$ at 220 nm, and $1 \times 10^{-3}$ at 180 nm. These yields are at least two orders of magnitude greater than those of the amino acids and more than three orders of magnitude greater than that of phytol over this wavelength region. Photoemission observed in chlorophyll thin films is due to the porphyrin moiety of the molecule. High contrast is obtained in photoelectron micrographs of chlorophyllin deposited on substrates of bovine serum albumin, dipalmitoyl phosphatidylcholine, or starch. Chlorophyll is expected to be the dominant photoemissive component of thylakoid membranes and accounts for the image contrast observed in photoelectron micrographs of spinach chloroplasts.

F-AM-C3 FLUORESCENCE DEPOLARIZATION STUDIES OF CHLOROPHYLL IN ORIENTED LIPID MULTILAYERS. R. Bansil[1],[2], L. Powers[3], and N.A. Clark*, Division of Engineering and Applied Physics, Harvard University, Cambridge, MA 02138

Oriented multilayers are formed from a mixture of chlorophyll a and dipalmitoyl-L-α-lecithin using a recently developed method (L. Powers and N.A. Clark (1975) Proc. Nat. Acad. Sci. 72, 840). These samples are several thousand bilayers in thickness and the water concentration can be varied from monohydrate to maximum hydration (~20%). The depolarization ratio $(p=(I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp}))$ is measured for various sample geometries and polarizations of the incident light. It is found that as the water content of the sample is increased the total intensity of fluorescence increases while the depolarization ratio decreases significantly. This result implies either a greater energy transfer at lower water concentrations or a better ordering of the chlorophyll molecules. The orientation of the chlorophyll with respect to the plane of the lipid bilayer, deduced from both absorption dichroism and fluorescence depolarization measurements, will also be discussed. (Supported by DMR 7302088, DMR 7302020 and Joint Services Electronics Program.)
1. Supported by Vinton Hayes Fellowship, Div. of Engineering and Applied Physics, Harvard University.
2. Present address - Harvard-M.I.T. Program in Health Sciences and Technology, M.I.T., Cambridge, MA 02139
3. Supported by N.I.H. Training Grant No. 5-T01 GM00782-15

**F-AM-C4** INTERACTIONS OF FLUORESCENT ANALOGS OF ADENINE NUCLEOTIDES WITH COUPLING FACTOR ISOLATED FROM CHLOROPLASTS. D.L. VanderMeulen and Govindjee, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

By monitoring fluorescence polarization, the binding of fluorescent-modified adenine nucleotides (εAMP, εADP, εATP) to isolated chloroplast coupling factor protein ($CF_1$) was investigated. Both εADP and εATP were found to bind, but εAMP showed no binding. The binding curves for εADP were shifted depending on the divalent cation, indicating that $Mg^{++}$ may be somewhat more efficient as compared to $Ca^{++}$. Phosphate, when added first, suppressed εADP binding, but produced an enhancement when added after the nucleotide. Unmodified ATP does not compete efficiently for the εATP binding site; rather, both ATP and ADP acting at low concentrations appear to slightly enhance ATP binding or stabilize a favorable protein conformation, or both. The polarization of fluorescence of the tyrosine residues intrinsic to $CF_1$ was measured as an independent indication of possible conformational changes important in phosphorylation. It was found that divalent cations produce a significant increase in polarization of protein fluorescence, with $Mg^{++}$ being somewhat more efficient than $CaCl_2$; both KCl and $KH_2PO_4$ produced only a small increase. The $Mg^{++}$ induced change in protein fluorescence polarization was shown to be reversible by addition of EDTA. Low concentrations of ADP or ADP + phosphate also produced a small increase in fluorescence polarization of tyrosine in CF, and the ADP effect was smaller when phosphate was added first.

Thanks are due to Professor Gregorio Weber for the use of his photon counting polarization instrument.

**F-AM-C5** THR PRIMARY ELECTRON ACCEPTOR OF CHLOROPLAST PHOTOSYSTEM I. Richard Malkin, Department of Cell Physiology, and Alan J. Bearden, Donner Laboratory, University of California, Berkeley, California 94720

A controversy has arisen about the identity of the primary electron acceptor in chloroplast Photosystem I. Earlier research supports the assignment of a membrane-bound iron-sulfur protein in this role (1,2) while the recent work of Evans et al. (3) suggests a different component which appears in electron paramagnetic resonance (EPR) spectra at 9°K after treatment of subchloroplast particles under strongly reducing conditions (illumination at pH 10 in the presence of dithionite and methyl viologen) where the iron-sulfur centers are reduced chemically. Using low-temperature photooxidation of P700 as a measure of photochemical activity, we have shown that more than 90% of $P700^+$ production in three different preparations with Photosystem I activity is concomitant with the photoreduction of a bound iron-sulfur protein. Furthermore, we could not find evidence for the new component by using low-temperature EPR techniques. Based on this experiment which shows a strong correlation between P700 photooxidation and bound iron-sulfur photoreduction and the finding that chemical reduction of the iron-sulfur protein blocks subsequent P700 photooxidation, it is our conclusion that a bound iron-sulfur protein center serves to accept an electron from reaction-center P700.

1. Malkin, R. and Bearden, A. J., Proc. Nat. Acad. Sci. (U.S.) 68, 16-19 (1971).
2. Bearden, A. J. and Malkin, R., Biochim. Biophys. Acta 283, 456-468 (1972).
3. Evans, M.C.W., Sihra, C. K., Bolton, J. R. and Cammack, R., Nature 256, 668-670 (1975).

(Research supported by the Energy Research and Development Administration and the National Science Foundation.)

**F-AM-C6** THE NATURE OF THE PRIMARY ELECTRON ACCEPTOR OF PLANT PHOTOSYSTEM II. Richard Malkin and David B. Knaff. Department of Cell Physiology, University of California, Berkeley, California 94720.

Treatment of Photosystem II fragments with the oxidant $K_2IrCl_6$ destroys approximately 50% of the bulk chlorophyll and results in fragments that are 2-fold enriched in P680 (the Photosystem II reaction-center chlorophyll) and cytochrome $b_{559}$. The fragments retain net Photosystem II electron-transport activity and a fully competent reaction center, as evidenced by P680 photooxidation and subsequent reduction in a back reaction with the primary electron acceptor ($t_{1/2}$ = 5 msec at 25°K). The $K_2IrCl_6$-treated fragments contain no photoactive or chemically detectable C-550 and do not exhibit any variable fluorescence. Because the Photosystem II primary electron acceptor appears to be unaffected by oxidant treatment, these results imply that neither C-550 nor the fluorescence-quenching substance functions as the primary electron acceptor of Photosystem II. Hexane extraction experiments suggest that the C-550 absorbance change may result from an electrochromic band-shift of membrane-bound carotenoids near the Photosystem II acceptor site. The identity of the Photosystem II primary electron acceptor itself is still unknown. However, indirect methods have made possible further characterization of its oxidation-reduction properties. The Photosystem II primary acceptor has a midpoint potential of -10 mV at pH 7 and a pH-dependence that indicates that one proton per electron is taken up on reduction. The reduced acceptor has a pK = 8.9. This pK value, taken in conjunction with recent kinetic studies, suggests that the acceptor of Photosystem II functions with an effective midpoint potential of -130 mV on the time-scale of photosynthetic electron transport.

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F-AM-C7 PHOTON-RATE DEPENDENT PHOTOCHEMISTRY OF CHLOROPLAST PHOTOSYSTEM I AT LOW TEMPERATURES. Alan J. Bearden, Donner Laboratory, and Richard Malkin, Department of Cell Physiology, University of California, Berkeley, California 94720

By using low temperature electron paramagnetic resonance spectroscopy at temperatures between 15°K and 77°K to measure the photooxidation of Photosystem I reaction-center chlorophyll (P700), a comparison was made of the photochemical effectiveness of equal numbers of incident photons ($3x10^{17}$) from a low-intensity light source (tungsten-iodine lamp and interference filter) and a high-intensity light source (flashlamp-pumped tuneable dye laser). In the low-intensity experiment light saturation was observed; that is, further illumination produced no further photochemical reaction. However when the same number of photons were furnished by the high-intensity light source only ~40% of the photooxidation of P700 produced in the low-intensity experiment was found. Identical effects were found with chloroplasts, sub-chloroplast particles (D-144), or with particles highly enriched in Photosystem I components (LDAO particles). The reduced photochemical effectiveness of the high-intensity illumination was independent of reaction-center chlorophyll to total chlorophyll ratio, independent of the wavelength of illumination from 650nm to 715nm, and independent of the temperature of the sample from 77°K down to 15°K. Subsequent flashes in the high-intensity experiment each produce the same partial conversion of the remaining unreacted sample. A low-intensity illumination after a laser flash completes the reaction of the remaining P700 with the same kinetics (photon-limited) as in an initial low-intensity experiment. The applicability of several models to this phenomenon based on considerations of antenna chlorophyll matrices and the properties of reaction centers will be presented. (Research supported by the Energy Research and Development Administration and the National Science Foundation.)

F-AM-C8 A MODEL FOR THE SPATIAL RELATIONSHIPS OF PHOTOSYSTEM I, PHOTOSYSTEM II AND THE LIGHT HARVESTING COMPLEX IN CHLOROPLAST MEMBRANES. P.A. Armond,* L.A. Staehelin,* and C.J. Arntzen, Department of Botany, University of Illinois, Urbana, Illinois 61801 and Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80302.

Previous studies in our laboratory [Biophys. J. 15(2): 223a, 224a (1975)] have demonstrated a close correlation between the presence of a light harvesting pigment-protein complex (LHC) and the presence of grana stacks and divalent cation regulated excitation energy distribution in chloroplast membranes. Pea plants which were grown in darkness for 7 days and then in intermittent light (2 min light, 118 min dark cycles) for 2 days contained no LHC and had no grana or $Mg^{++}$ regulation of "spillover". Freeze-fracture examination of these same membranes revealed a reduction in size of the membrane subunits. The particles of the EF fracture face, associated with Photosystem II, as well as particles of the PF fracture face, associated with Photosystem I, show a reduction in size, although the density of the particles is similar to that found in experimentally unstacked lamellae of normal chloroplast membranes. Transfer of intermittent light plants to continuous light results in synthesis of LHC and an increase in size of the freeze-fracture particles. Aggregation of the EF face particles into regions of grana stacking coincides with the appearance of LHC. A model for PS II - PS I - LHC interaction, based on development and distribution of the various size classes of the freeze-fracture particles will be presented.

F-AM-C9 DYNAMIC INTERACTIONS BETWEEN PIGMENT-PROTEIN COMPLEXES IN CHLOROPLASTS. C.J. Arntzen, P.A. Armond,* and C.H. Ditto* (Intr. by S. Helman), Department of Botany, University of Illinois, Urbana, Illinois 61801

The effects of monovalent and divalent cations upon detergent fractionation of chloroplasts was determined. In "low-salt" chloroplasts, both photosystem I and II activities were recovered in the supernatant fraction following a 40,000 x g centrifugation of digitonin-treated lamellae.

The supernatant had a Chl a/b ratio equal to that of the control chloroplasts. A photochemically inactive pigment-protein complex (light harvesting complex; LHC) with a Chl a/b ratio of 1.3 could be further purified from this "light" preparation by sucrose gradient separation. Detergent fractionation in the presence of cations resulted in recovery of all PS II activity in a "heavy" fraction (40,000 x g pellet) with a Chl a/b ratio of 1.8. The cation concentration dependency for generating a "heavy" PS II was the same as that needed for regulation of excitation energy distribution between PS I and II (assayed by fluorescence techniques). We speculate that "spillover" control is mediated by salt-induced changes in interactions between the LHC and the PS II complex within the membrane. This hypothesis will be shown to be consistent with freeze-fracture studies of chloroplast membranes in the presence or absence of cations.

**F-AM-C10** NMR STUDIES ON PHOTOSYNTHESIS: PROTON RELAXATION AS A MONITOR OF MEMBRANE-BOUND MANGANESE AND OF THE CHARGE ACCUMULATING STATES DURING OXYGEN PRODUCTION. T. Wydrzynski, N. Zumbulyadis*, P.G. Schmidt, and Govindjee, Departments of Physiology and Biophysics and Chemistry, University of Illinois, Urbana, Ill. 61801

A large component of the water proton spin-lattice relaxation rate ($1/T_1$) in chloroplasts can be attributed to membrane-bound manganese (Mn). Release of Mn either by Tris washing (0.8 M, pH 8.0) or $NH_2OH$-EDTA extraction reduces $1/T_1$ to 40% of the control. Addition of an oxidant (potassium ferricyanide) to untreated chloroplasts leads to a small decrease while a reductant (tetraphenylboron or $NH_2OH$) leads to as much as a 3-fold increase in $1/T_1$. Since the reductants have no effect in Tris-washed chloroplasts, the redox reagents are probably affecting the bound Mn. The $1/T_1$ is a function of chloroplast activity; it decreases as the rates of photoreduction of a Hill oxidant decreases. In continuous light, untreated chloroplasts show a reversible net decrease in $1/T_1$. In a sequence of brief flashes the spin-spin relaxation rate ($1/T_2$) [where $1/T_2 \geq 1/T_1$] shows a damped oscillatory pattern with a period of four and peaks after the 3rd, 7th, 11th and 15th flashes. This strongly suggests that the proton relaxation also monitors the charge accumulating states in Kok's model for oxygen evolution which, therefore, may be directly related to the membrane-bound Mn.

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**F-AM-C11** EFFECTS OF IMIDOESTER CROSSLINKING ON STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF ISOLATED SPINACH CHLOROPLASTS. J. Isaakidou, and G. Papageorgiou*, Department of Biology, Nuclear Research Center Democritos, Athens, Greece.

Isolated spinach chloroplasts were incubated with the bifunctional crosslinking agent dimethyl suberimidate at 0° to 4°C. The treatment partially inhibits methylviologen-mediated photooxidation of water, but not photooxidation of the artificial electron donor diphenylcarbazide. The photooxidation of water reaches a low steady level within minutes of exposing the chloroplasts to the imidoester. On the other hand, the osmotic response of the chloroplasts stabilizes at a lower level after 3 to 4 h of incubation. Threshold ratios for stabilized osmotic response lie in the range of 15-20 μmoles imidoester/mg Chl, whereas for stabilized photooxidation of water in the presence of methylviologen they are in the range of 2-5 μmoles imidoester/mg Chl. These results imply a specific inhibition by the imidoester between the water and the diphenylcarbazide photooxidation sites. The inhibition does not appear to be directly linked to the immobilization of the thylakoid membranes. Low level crosslinking (5-10 μmoles imidoester/mg Chl) was found to protect the chloroplasts against loss of activity during prolonged storage at 0° to 4° C.

**F-AM-D1** INTERACTION OF MELANIN WITH PARAMAGNETIC COPPER: A DILEMMA RESOLVED. H.M. Swartz, T. Sarna*, J.S. Hyde*, Radiation Biology and Biophysics Department, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Melanins are complex, variable polymers which are very widely distributed in animals. They possess unpaired electrons that are generally very unreactive, but which apparently interact with high concentrations of paramagnetic ions such as $Cu^{2+}$, $Fe^{3+}$ and $Mn^{2+}$ ions. (Blois, M.S., Zahlan, A.B. and Maling, J.E., Biophys. J. 4 (1964), 471). This apparent reactivity with copper is anomalous, because the melanin free radical is stable to very severe physical treatments such as boiling for 24 hours in concentrated acid. When melanin does react chemically, even with other free radicals, it is its quinoid structure that actually reacts, while the unpaired electron concentration remains unaffected. (Swartz, H.M., p179 in Biological Applications of Electron Spin Resonance, Swartz, H.M., Bolton, J. and Borg, D. eds., 1972). We have now resolved this apparent paradox. We hypothesized, in analogy with a theory of Leigh (J. Chem. Phys. 52 (1970), 2608), that the apparent reaction of melanin free radicals with paramagnetic copper was in fact a purely physical consequence of extreme line broadening arising from the copper-magnetic-dipole--melanin-electron-magnetic-dipole interaction. In this report we shall describe the results of experiments with paramagnetic shift reagents, variable temperatures, variable microwave powers and variable microwave frequencies that confirm our hypothesis. These results have significant implications as to the interpretation of ESR experiments in biological systems in which copper or other paramagnetic ions are present. These physical interactions of the magnetic dipoles should be useful probes of complex biomolecules in regard to the nature and sites of both free radicals and paramagnetic metal ions that are bound to the biomolecules. Melanin has strong ion exchange properties which we are now studying with this technique.

**F-AM-D2** SPECTROPHOTOMETRIC DETECTION OF HYALURONIC ACID - $Cu^{2+}$ COMPLEX: B. Chakrabarti, N. Figueroa* and B. Nagy. Retina Research, Eye Research Institute of Retina Foundation, Boston Biomedical Research Institute and Department of Neuropathology, Harvard Medical School, Boston, Massachusetts 02114.

While the depolymerization of hyaluronic acid (HA) by $Cu^{+}$ or the catalytic activities of $Cu^{2+}$ in the presence of autoxidants have been well documented, the mechanism of this degradation reaction is not well understood. It has been suggested that only the $Cu^{+}$ interacts with HA in the depolymerization process. Spectrophotometric investigations have been undertaken to study the interaction properties of $Cu^{2+}$ with HA. Binding of $Cu^{2+}$ to HA is indicated by difference spectral changes in the ultraviolet. Two peaks at 276 and 223 nm appear as $Cu^{2+}$ binds to HA. Titration with $Cu^{2+}$ using the absorption peaks indicate that the metal ion binds with respect to disaccharide unit of the polymer in 1:1 molar ratio at 0.1 M NaCl solution. The absorption values of the respective peaks calculated on the basis of disaccharide unit are 100 and 240. The absorption peaks decrease with increasing ionic strength of added NaCl. On overnight standing, the shape and magnitude of the absorption bands remain unchanged and no degradation of the polymer can be detected from the intrinsic viscosity measurements.

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**F-AM-D3** PURIFICATION AND PROPERTIES OF A CALCIUM-BINDING PROTEIN FROM HUMAN BLOOD PLATELETS. E.B. McGowan, S. Speiser*, and A. Stracher, Dept. of Biochemistry, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203

A high-affinity calcium-binding protein (CaBP) has been purified to homogeneity from a high salt-3% n-butanol extract of human blood platelets. The protein is the major acidic protein in platelets. Its molecular weight is approximately 19,000 daltons on SDS-polyacrylamide gels. Its preliminary amino acid composition indicates that it is highly acidic, contains no tryptophan or cysteine, and has a high phenylalanine:tyrosine ratio. The amino acid composition is not identical to any other reported CaBP; it most closely resembles a phosphate-containing CaBP from brain. The platelet protein is not sensitive to treatment with alkaline phosphatase either in electrophoretic mobility or in calcium-binding capacity. The protein binds 1-2 moles of calcium per mole of protein with a dissociation constant of about 1 uM; magnesium and sodium are not good competitive inhibitors. The subcellular localization of the platelet binding protein is being determined using antibodies raised in rabbits; cross-reactivity of the platelet protein with antibodies to a variety of other CaBPs is being determined. No function for the platelet CaBP has yet been determined.

Supported by Grant Number HL14020 from the National Heart and Lung Institute, NIH, and by the New York Heart Association.

**F-AM-D4** SECONDARY STRUCTURE OF LIGHT MEROMYOSIN CHARACTERIZED BY RAMAN SPECTROSCOPY. E.B. Carew*, J.C. Seidel, E. Stanley and J. Gergely, Dept. Muscle Research, Boston Biomed. Res. Institute; Dept. Neurology, Mass. Gen. Hosp.; Dept. Neurol. and Biol. Chem., Harvard Med. Sch.; Harvard-MIT Program in Health Sciences; Boston, Mass., and U. Michigan, Dearborn

Raman spectra of light meromyosin (LMM) and tropomyosin (TM) exhibit a broad band of activity between 1260 and 1360 $cm^{-1}$ arising from overlapping CH bending modes and a combination CN stretch-NH bending mode of the peptide bond. Other studies on amides of peptide and protein show that NH related peaks in this spectral region are assignable to $\alpha$ helices while Raman activity in the 1250 to 1220 $cm^{-1}$ band is associated with random coil and pleated $\beta$ structures. On LMM the 1260-1360 $cm^{-1}$ band is characterized by a triplet at 1304, 1318, 1340 $cm^{-1}$ with each member of approximately equivalent intensity. On LiCl denaturation of this $\alpha$-helical protein the 1304 $cm^{-1}$ peak disappears and is replaced by another broad peak centered at 1244 $cm^{-1}$; in $D_2O$ this 1304 $cm^{-1}$ peak shifts approximately 350 $cm^{-1}$ to a lower frequency characteristic of the ND combination mode. We thus conclude that the 1304 $cm^{-1}$ peak of LMM behaves as an amide III mode and is attributable to $\alpha$ helical structure. The spectrum of TM also shows a triplet in the 1260-1360 $cm^{-1}$ region; its 1304 $cm^{-1}$ peak likewise is displaced on denaturation by LiCl or deuteration. In LMM and myosin the intensity of the 1260-1360 $cm^{-1}$ band is enhanced by increasing the protein concentration or by aggregation in 0.138M KCl, 2 mM Tris pH 8.4. Since the peaks at 1318 $cm^{-1}$ and 1340 $cm^{-1}$ decrease in amplitude by 70-80% in $D_2O$ while the 1304 $cm^{-1}$ peak completely disappears on deuteration, we assign CH bending activity to the 1318, 1340 $cm^{-1}$ peaks with the major contributions to the 1260-1360 $cm^{-1}$ band arising from 1304 $cm^{-1}$ amide III.

(Supported by grants from NIH, NSF, AHA, MDAA and the Research Corporation)

**F-AM-D5** PREVALENCE OF $\alpha$-HELICAL FORM IN AVIAN LENS PROTEINS AS STUDIED BY RAMAN SPECTROSCOPY. Nai-Teng Yu, E. J. East*, School of Chemistry, Georgia Institute of Technology, Atlanta, Ga. 30332 and J.F.R. Kuck, Jr.*, Department of Ophthalmology, Emory University, Atlanta, Ga. 30322.

The avian lens is unlike other vertebrate lenses in several respects: it contains a unique crystallin, FISC or $\delta$-; it does not develop cold cataract and is generally less susceptible to other types of cataract; it is extremely soft with no distinct nucleus; it has no grossly visible fluorescence; it has relatively low amounts of $\gamma$-crystallin.

We present evidence based on Raman spectroscopy of the intact, capsulated living lens that the FISC proteins of the chick and pigeon lens exist chiefly in the $\alpha$-helical conformation. This with the low protein content is almost certainly the reason for the soft consistency of the bird lens which must be easily deformable to allow the rapid accomodation necessary to the vision of birds, especially raptors.

The Raman spectrum of a human fetus lens (~6 months) indicates that there is little or no $\alpha$-helix present. The human lens proteins are mixtures of $\beta$-pleated and random-coiled structures.

The Raman signal due to thiol groups appears near 2580 $cm^{-1}$. It is quite strong in human lens, but completely absent in the spectrum of chick lens. Thus the occurence of this low thiol protein ($\delta$-crystallin) in the $\alpha$-helical form may explain the failure of the bird lens to develop cold cataract or a firm nucleus.

**F-AM-D6** NORMAL VIBRATION ANALYSIS OF BETA POLYPEPTIDES. W. H. Moore and S. Krimm, Biophysics Research Division and Department of Physics, University of Michigan, Ann Arbor, Mich. 48104

A valence force field has been refined to explain the infrared and Raman spectra of polyglycine I (PGI) in a rippled sheet structure and poly-(L-alanine) (PLA) and poly-(L-analylglycine) in a pleated sheet. Normal coordinate calculations for these beta polypeptides have been done with this self consistent force field, which includes the effects of intermolecular hydrogen bond interactions and transition dipole coupling. The frequency splittings that result from the interaction between transition dipoles are calculated and shown to be affected by interactions within a given sheet and between different sheets. This approach enables us to predict the $\nu(\pi,\pi)$ Amide I frequency, which is generally not observed, and the frequencies of the four Amide II modes associated with each polypeptide. By using observed intensities to calculate unperturbed Amide A and B vibrations, it is possible to verify predicted frequencies of the $\nu(\pi,\pi)$ Amide II modes. Moreover, the higher frequency of the unperturbed Amide A mode in PGI (3286 $cm^{-1}$) as compared to PLA (3242 $cm^{-1}$) implies a weaker hydrogen bond in the former. The weaker H···O bond is consistent with the differences in crystal structure between the two polypeptides, i.e., rippled PGI and pleated PLA. Our low frequency predictions also agree well with observations. However, contrary to the presently accepted assignment, the far infrared band between 100 and 130 $cm^{-1}$ is not a translatory lattice mode, but is basically intramolecular in nature, although it is affected by the relative motion of adjacent chains. This research was supported by NSF grants BMS74-21163 and MPS75-05239.

**F-AM-D7** COHERENT ANTI-STOKES RAMAN SCATTERING. Bruce S. Hudson, Department of Chemistry, Stanford University, Stanford, CA 94305

Coherent anti-Stokes Raman scattering spectroscopy (CARS) is a nonlinear optical technique for obtaining Raman spectra with several significant advantages over conventional resonance enhanced Raman scattering. For dilute solutions of biological macromolecules the principal advantages are (1) complete rejection of fluorescence (2) signal to noise enhancement of $10^8$ and (3) lower average power levels are necessary. Experimental demonstrations of resonance enhanced CARS will be discussed with particular reference to the choice of optimum experimental conditions. Problems associated with the nonresonant background signal are often not important under conditions of resonance enhancement. The theory of CARS will also be reviewed with emphasis on resonance enhancement.

**F-AM-D8** LOW FREQUENCY COLLECTIVE MODES OF POLYINOSINIC * POLYCYTIDYLIC ACID. C. P. Beetz, Jr. and G. Ascarelli‡ Physics Department, Purdue University, West Lafayette, Indiana 47907

The optical absorption of Poly I * Poly C films at room temperature shows structure in the far infrared where $f < 100\ cm^{-1}$. Although the overall absorption resembles that of water a distinct feature that has a maximum at $\sim 45\ cm^{-1}$ stands out when the sample is in the A-conformation. This maximum shifts to $\sim 70\ cm^{-1}$ when the sample is in the A'- conformation. The water-like background cannot be quantitatively explained. At all values of relative humidity the background absorption coefficient is about 3 times larger than that expected from the amount of water present in the film as determined from a gravimetric study. The structure observed at $\sim 45\ cm^{-1}$ in the A-conformation approximately coincides with the region where a large number of intense lines are predicted from the collective mode calculation of Eyster and Prohofsky[1]. (Supported in part by NIH grant GM00779.)

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**F-AM-D9** CORRECTED LOW-ANGLE X-RAY INTENSITIES FOR COLLAGEN. S. K. Wang and C. R. Worthington, Departments of Biological Sciences and Physics, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

In the study of biological structure by x-ray diffraction the observed intensities are multiplied by a correction factor C(h) to obtain the correct Fourier transform. Our analysis (Abstract, Biophysics Soc. 1975) for a cylindrical specimen with an axial repeating structure shows that C(h) is a function of the relative values of $R_o$(the radius of reciprocal disc), $\omega$ (the mean angle of disorientation) and $\epsilon$ (the mean angle of beam divergence). Correction factors for rat tail tendon collagen and dogfish fin elastoidin have been calculated theoretically and measured experimentally. For the theoretical C(h), the values of $R_o$ and $\omega$ were obtained from pinhole and tilting experiments and then used in our theoretical expressions. For the experimental C(h), the diffraction intensities from a stationary sample were compared with those from an oscillating sample - the latter gave the correct intensities directly. Our results show good agreement between theory and experiments. In both collagen and elastoidin cases, it is found that the high order reflections have a more important role in controlling the shape of the Fourier syntheses than used in previous work. The Patterson functions calculated using the corrected intensity data have the same general shape as shown in earlier publications but the minor peaks are now comparatively resolved or magnified.

**F-AM-D10** SMALL-ANGLE X-RAY SCATTERING STUDIES ON 20S AND 13S PRODUCTS OF α AND β HELIX POMATIA HEMOCYANIN. A. Kent Wright and John E. Baxter*, Department of Biochemistry, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

A structural model of Helix pomatia hemocyanin composed of 180 identical spherical subunits has been proposed which agrees with data based on the following methods; electric birefringence decay, viscosity, sedimentation, electron microscopy, three-dimensional image reconstruction, and small angle X-ray scattering (1). Symmetries within this model suggest several different modes of dissociation. Sedimentation coefficients, radii of gyration, and low angle X-ray scattering curves have been calculated for these different models and comparisons with experimental observations of these parameters will be presented. Sedimentation coefficients and low angle X-ray scattering curves have been obtained for the dissociation products of α and β H. pomatia hemocyanin in 0.1 M Tris-HCl, 0.5 M NaCl, at two pH values, 7.9 and 10.4. Additionally, the X-ray scattering results reported here for α H. pomatia hemocyanin will be compared with those reported by Pilz and coworkers (2,3,&4).

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**F-AM-D11** NANOSECOND EMISSION ANISOTROPY OF MONOAMINE OXIDASE. John B. Massey* and Jorge E. Churchich, Department of Biochemistry, University of Tennessee, Knoxville, Tennessee 37916

Two fluorescent probes, the dye ANS and the substrate kynuramine, were used to study nonpolar binding sites in the enzyme monoamine oxidase from pig plasma. Nanosecond emission anisotropy measurements were performed on the ANS-enzyme complex to determine whether the dimeric enzyme displays any nodes of flexibility in the nanosecond range. The two quantities calculated from nanosecond measurements S(t) and D(t) decay in a monoexponential manner with decay times $\zeta_S$ = 10.2 and $\zeta_D$ = 9.3 nanoseconds. The observed rotational correlation time ($\theta$ = 105 nanoseconds) agrees well with the value expected for a dimeric protein of 180.000 molecular weight. No dissociation of the dimer into monomers could be detected in the nanosecond range. The fluorescence enhancement that follows the addition of the substrate kynuramine to the enzyme was used to investigate the environment of the catalytic binding site under anaerobic conditions. Two catalytic binding sites characterized by a dissociation constant $K_D$ = 10μm were detected by fluorometric titrations. The frequency shift of the absorption and fluorescence transitions of bound kynuramine could be related to the presence of a polar-environment at the catalytic binding site.

**F-AM-D12** CHARACTERIZATION OF MACROMOLECULAR STRUCTURE BY DIFFUSION MEASUREMENTS. M. E. McDonnell and A. M. Jamieson, Department of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106.

Diffusion coefficients measured by quasielastic light scattering are shown to be useful probes of branching in biological molecules. A method previously described (1) which combines diffusion and viscosity measurements is used to determine the molecular weight of the glycoprotein ovomucoid. When the molecule is denatured in 6M guanidine hydrochloride and 0.1M mercaptoethanol the diffusion coefficient is much smaller than would be predicted for a linear molecule of that weight, but it is consistent with chain branching. These observations are in agreement with previous measurements of saccharide hydrolysis rates (2). A procedure to determine effective segment lengths of macromolecules in a random coiled conformation and to describe the deviation of the solution from ideal behavior using diffusion measurements is also proposed. This analysis is applied to denatured proteins and polysaccharides. This work was sponsored by USPHS under grant number AM-17110-08.

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**F-AM-D13** CHARACTERIZATION OF EXPONENTIAL AND POISSON MOLECULAR LENGTH DISTRIBUTIONS FROM ELECTRICAL BIREFRINGENCE DECAY. M. M. Judy*, G. A. Campbell*, and R. M. Dowben, Biophysics Department, University of Texas Health Science Center, Dallas, Texas 75235.

Electrical birefringence is the change in transmittance of plane-polarized light caused by orientation of electrically and optically anisotropic molecules in an electric field. Decay of the electrical birefringence upon instantaneous removal of the orienting field depends upon the length of long rigid macromolecules, and thus, contains information about molecular length distribution. For a continuous length distribution, the birefringence decay $\Delta n(t)$ normalized to the steady state value $\Delta n_o$ is given by

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

where integration is over molecular length, $\theta(\ell)$ is the rotational diffusion constant of a molecule length $\ell$, and $\Delta n\,(\ell)\, d\ell$ is the contribution to birefringence of species having lengths between $\ell$ and $\ell + d\ell$ and is proportional to the volume fraction of molecules in the length range. For length distributions of the form $h(\ell) = \ell^s \exp[(\ell-a)/b]$, $s = 0$ and $s \neq 0$, respectively, which correspond to the equilibrium distribution of a linear polymer and the meta-stable distribution formed by addition of monomer to pre-existing nuclei, we have derived equations for

$$[d^n(\Delta n(t)/\Delta n_o)/dt^n]_{t=0} = \int [\theta(\ell)]^n\, \Delta n_o(\ell) d\ell / \int \Delta n_o(\ell)\, d\ell \qquad (2)$$

with $n > 0$, and for

$$\int_0^\infty (\Delta n(t)/\Delta n_o) dt = \int [\theta(\ell)]^{-1}\, \Delta n_o(\ell) d\ell / \int \Delta n_o(\ell)\, d\ell \qquad (3)$$

which is the area (A) underneath the decay curve. Equations (2) and (3) depend upon the values of s, a, and b and show that knowledge of (A) and the first two derivatives are sufficient to determine s, a, and b uniquely. Currently available fast photo-detector and field-switching technologies allow characterization of these length distributions through evaluation of the derivatives and area of the birefringence decay curve.

**F-AM-D14** THE USE OF MOMENT INDEX DISPLACEMENT IN ANALYZING FLUORESCENCE TIME DECAY DATA. Enoch W. Small* and I. Isenberg, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331.

Moment Index Displacement (MD) automatically corrects a number of significant nonrandom instrumental errors in fluorescence time decay measurements. Three component data, obtained by measuring the fluorescence decay of three different species mixed in the same solution, was used as a test sample. The use of MD with the method of moments enabled us in all cases to recover the correct parameters in a three component analysis of the data. Using real data, it was shown, as predicted by theory, that MD corrects three nonrandom instrumental errors: (1) the presence of scatter in the data, (2) time origin shifts between lamp and fluorescence data, and (3) lamp drift, or time dependent changes in the shape of the excitation curve. The ability to analyze fluorescence decay data is limited by nonrandom or consistent errors, rather than by random statistical fluctuations. Our data shows that the use of the method of moments with MD to analyze fluorescence decay data is not a curve-fitting procedure. This procedure will accurately obtain decay parameters for multiple exponential decays from certain badly distorted data, yielding a calculated curve very different from the actual data. Recently we have shown that, in practical cases, for a macromolecule of general shape, the fluorescence anisotropy will decay as a sum of one, two, or at most three exponentials, and that these exponentials can be used to uniquely determine the three different semi-axes of an ellipsoid approximating the shape of the macromolecule.

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**F-AM-E1** ELASTIC, AREA COMPRESSIBILITY MODULUS OF RED CELL MEMBRANE. E.A. Evans*, R. Waugh*, and L. Melnik*, Department of Biomedical Engineering, Duke University, Durham, N.C. 27706. Sponsored by J.M. Corless, Department of Anatomy, Duke University.

Micropipette measurements of isotropic tension versus area expansion in preswollen single human red cells gave a value of 288 + 50 S.D. dynes/cm for the elastic, area compressibility modulus of the total membrane at 25° C. This elastic constant, characterizing the resistance to area expansion or compression, is about 4 x $10^4$ times greater than the elastic modulus for shear rigidity, 7 x $10^{-3}$ dynes/cm. The tension was found to be linear and reversible for the range of area changes observed (within the experimental system resolution of 10%). The maximum fractional area expansion required to produce lysis was uniformly distributed between 2 - 4% with 3% average and 0.7% S.D. By heating the cells to 50° C, it appears that the structural matrix (responsible for the shear ridigity and most of the strength in isotropic tension) is disrupted and primarily the lipid bilayer resists lysis. Therefore, the relative contributions of the structural matrix and lipid bilayer to the elastic, area compressibility could be estimated. The maximum isotropic tension at 25° C is 10 - 12 dynes/cm and at 50° C is between 3 - 4 dynes/cm. From this data, the respective compressibilities are estimated at 193 dynes/cm and 95 dynes/cm for structural network and bilayer. The latter value correlates well with data on in-vitro, monolayer surface pressure versus area curves at oil-water interfaces.

**F-AM-E2** A.SPIN LABEL STUDY OF ERYTHROCYTE MEMBRANES DURING HYPEROSMOTIC STRESS M.A.Nunes(1)*C.F.Chignell(2),H.T.Meryman(3).Department of Physiology,Instituto de Ciências Biomédicas,U.S.P.,S.P.,Brasil(1).National Heart and Lung Institute,Bethesda,Md. 20014(2).American National Red Cross,Bethesda,Md.20014(3).

Using stearic acid spin labels I(12,3) and I(1,14) no detectable changes were found in the polar head or tail regions of membrane phospholipids when intact human red cells were exposed to hypertonic solutions of sodium chloride ranging from 295 to 3055 mOsm (pH 7.4). By using a more sensitive spin label, androstane, which is able to reveal small changes in the environment ("intermediate immobilization"), an increase of the correlation time of about 28 percent was detected in intact human red cells at 3055 mOsm. This finding was taken to indicate a decrease in fluidity of the membrane phospholipids as the concentration of the medium increased. Conformational changes of the red cell ghost membrane proteins were studied with maleimide (MSL) and iodoacetamide (ISL) spin labels. With MSL two components in the spectrum have been identified. A decrease of 36 percent at 3055 mOsm was observed in the amplitude ratio with MSL,while with ISL, which labels only SH groups, there was evidence of a strongly immobilized fraction. Our observations suggest that, in hyperosmotic NaCl, an increased rigidity of the red cell membrane develops, probably due to a decrease in fluidity of the phospholipids moieties accompanied by conformational changes of the red cell membrane proteins. Whether this is the result of osmotic gradients or simply due to the increase in ionic strength remains to be determined.

(1) Post-doctoral fellow: FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SAO PAULO.

**F-AM-E3** ALTERATION OF MEMBRANE CHARGE OF RBC. A. ZELMAN, Center for Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY. 12181

Uremic patients with high urea conc. and sickle cell disease patients undergoing HCNO therapy exhibit either high cellular $Na^+$ in RBC or slowed conduction neuropathy. HCNO is in equilibrium with urea. These effects indicate that HCNO is decreasing membrane positive charge by the following mechanism. $R\text{-}NH_2$ + HCNO → $R\text{-}NHCONH_2$ followed by $R\text{-}NH_3^+$ → $R\text{-}NH_2$ + $H^+$. The reaction of cyanate with membrane protein removes the amine group. The ammonium group dissociates $H^+$ inorder to re-establish equilibrium. The loss of positive charge tends to increase the permeability of $K^+$ and $Na^+$. Freshly drawn human blood was placed in an isotonic solution composed of Na, K, Mg, Ca, $PO_4$ Cl, glucose and $CNO^-$ ranged from 0.1 to 50 mM. Solutions were identical except that Cl was replaced with CNO. RBC were incubated at 37°. Na and K were determined by flame photometry. Our permeability experiments demonstrate that: 1) Cyanate selectively increases Na passive permeability and to a less extent increases K passive permeability. 2) Incubation of RBC's with CNO causes them to gain cellular Na while maintaining normal K levels. 3) The stoichiometry between Na and K active transport is not altered by cyanate. If cyanate would cause an accumulation of Na inside nerve cells as it does in RBC, the resting membrane potential would not be affected, but the magnitude of the action potential spike would be lessened; therefore the conduction velocity would be smaller. Thus the uremic state and the cyanate therapy state seem to be related via the urea-cyanate equilibrium and that chemical alteration of the membrane charge is responsible. reference: Zelman, A. et al Proceeding, Clin. Dial. Trans. Forum 4:172-179 (1974).

**F-AM-E4** ELECTROPHORETIC HOMOGENEITY OF HUMAN ERYTHROCYTES: STUDIES OF OLD AND YOUNG CELLS. S.J. Luner and D. Szklarek*, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024.

Studies of erythrocytes in an Endless Belt Electrophoresis apparatus in 0.013 M tris-acetic acid buffer, pH 7.4, containing 5% sucrose to prevent osmotic lysis show that the cells migrate electrophoretically as a homogenous population with a streak width no greater than 5% of the total distance of electrophoretic migration. Since this observation conflicted with published reports of significant loss of electrophoretic mobility depending upon the length of time spent in the circulation, separated fractions of old and young erythrocytes were studied electrophoretically. Centrifugation through mixtures of dimethyl phthalate and dibutyl phthalate fractionated the erythrocytes on the basis of density. Fractions containing 2-6% of the cells with the highest and lowest densities, representing the oldest and youngest cells respectively were used. To confirm that the cells were separated on the basis of age levels of intracellular aspartate aminotransferase activity were determined spectrophotometrically. The oldest cells contained $\frac{1}{3}$ to $\frac{1}{5}$ times the enzyme activity of the youngest cells, while the unfractionated cells showed an intermediate value. Electrophoresis of mixtures of the oldest and youngest cells yielded streaks no wider than those formed by the individual fractions, demonstrating that electrophoretic mobility is independent of cell age. Supported by Grant Number CA 13955 from the National Cancer Institute, DHEW.

**F-AM-E5** INTERACTION OF POLY-(L-LYSINE) WITH HEMOGLOBIN-FREE HUMAN ERYTHROCYTE MEMBRANES. L. C. McCaughan, J. B. Stamatoff,† and S. Krimm, Biophysics Research Division and Department of Physics, University of Michigan, Ann Arbor, Mich. 48104

Previous work has shown that neuraminidase-treated hemoglobin-free human erythrocyte membranes can be aggregated by poly-(L-lysine), PLL, small angle X-ray diffraction patterns of these aggregates exhibiting a 67 A periodicity (Stamatoff, Krimm, and Harvie, Proc. Nat. Acad. Sci. 72, 531 (1975)). We now find that untreated membranes can be aggregated with varying concentrations of PLL. Sealed ghosts were also aggregated by PLL, but could not be collapsed even by ultracentrifugation. PLL-treated ghosts, both sealed and unsealed, appear intact when viewed under phase contrast microscopy prior to ultracentrifugation. Only a very small fraction of whole erythrocyte cells are hemolyzed by PLL, and no detectable quantities of protein are released from the ghosts by the polypeptide. Small angle X-ray diffraction patterns of completely wet PLL-bound membranes give two unoriented orders of a 69 A periodicity. This periodicity shows small but monotonic variation with PLL concentration. Calculations based on a finite number of diffracting units show that approximately 13 lamellae comprise the diffracting domain. These results support a model composed of a periodic array of intact membranes about 55 A thick cross-linked by PLL layers about 12 A thick. We also find that PLL interferes with the binding of hemoglobin to ghosts, and will displace this protein from hemoglobin-bound ghosts. This result, plus the behavior of the sealed vesicles, strongly indicates that phase separation is not a relevant factor in our membrane preparations, thus supporting our earlier conclusion that the membrane is about 55 A thick. This research was supported by NSF grant BMS74-21163.
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**F-AM-E6** EXPERIMENTAL EVIDENCE THAT THE ERYTHROCYTE GLUCOSE TRANSPORT SYSTEM IS A PORE (NOT A CARRIER) MECHANISM. R.J. Bowman* and D.G. Levitt* (Intr. by J.A. Johnson), Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Glucose transport across the red cell membrane is usually interpreted in terms of a general carrier model. However, since transport through a pore that undergoes a conformational change and carrier transport are described by exactly the same set of kinetic equations, it is impossible to distinguish between a pore and carrier model from the transport kinetics. If glucose transport involved a pore that contained a specific glucose binding site, one would predict that any molecule the size of glucose or smaller should be able to pass through that pore nonspecifically. It had been shown previously that a large fraction of the erythritol (4 carbon) permeability was inhibited by glucose and it seems reasonable to assume that this glucose dependent fraction is using the glucose transport mechanism. We have verified this result for erythritol and have determined the glucose dependent permeability of 5 carbon (D and L arabitol and xylitol) and 6 carbon (mannitol) polyalcohols for the human erythrocyte. The glucose dependent permeability ($10^{-8}$ cm/sec) of the 4, 5 and 6 carbon compounds is 6, 0.2 and .02 respectively. The decrease in permeability with increasing molecular size is just what would be expected for a pore mechanism. Although these results could also be explained by a carrier mechanism in which the permeability is a measure of the relative affinity of the carrier, one would not expect the carrier affinity to have this simple size dependence.

F-AM-E7 PHOTOINACTIVATION OF GLUCOSE TRANSPORT IN HUMAN ERYTHROCYTES. Robert A. Farley, Kim D. Collins* and William H. Konigsberg,* Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Conn. 06510.

The transport of glucose across the human erythrocyte membrane is mediated by a membrane-bound protein which has not yet been isolated and characterized. Quercetin (3,3',4,5,7-pentahydroxyflavone), a compound found widely distributed in the plant kingdom, has been found to be a potent reversible inhibitor of glucose transport in human erythrocytes. The inhibition is characterized by a $K_I$ of $1.3 \times 10^{-5}$ M at pH6 and 20°C using the sorbose influx assay of Levine et. al. (BBA 225: 291, 1971), and is found to follow simple, linear non-competitive kinetics when examined using the glucose efflux assay of Karlish et. al. (BBA 255: 126, 1972). Martyr and Benisek (Biochem. 12: 2172, 1973) have shown that $\alpha,\beta$-unsaturated ketones are potential photoaffinity reagents. The inhibition of glucose transport in human erythrocytes becomes irreversible if the erythrocytes and quercetin are irradiated together using a light source with the main output in the region of 375 nm, corresponding to one of the absorption maxima of quercetin. Inactivation can be prevented by high concentrations of D-glucose, but not D-mannitol, in the irradiated solution. Supported by USPHS grants 1F32GM05199-01, 5R01AM18368, and HL16126.

F-AM-E8 MEMBRANE POTENTIALS IN HUMAN RED BLOOD CELLS DUE TO PROTON GRADIENTS. T.J. Callahan* and J.F. Hoffman, Dept. of Physiology, Yale University School of Medicine, New Haven, Conn. 06510.

Membrane potentials were estimated by fluorescent changes of the photosensitive cyanine dye, Di-S-$C_3$(5), with an Aminco-Bowman Spectrophotofluorometer. The addition of HCl or NaOH in a isotonic salt solution to red blood cells suspended in an unbuffered NaCl solution can cause significant depolarization or hyperpolarization respectively. Sufficient NaOH added to raise the external pH from 7.1 to pH 9.0 can cause an estimated 15-20 mV hyperpolarization change. Addition of sufficient acid to change the external pH from 7.1 to pH 6.2 caused a depolarization of a similar magnitude. The change in membrane potential vs pH appears linear within the range pH 6.7 to 8.5 and suggests that the red blood cell membrane behaves as a hydroxyl electrode (proton electrode) under these conditions. The rate and magnitude of similar changes in membrane potential of red blood cells suspended in NaCl-glycylglycine solution can be increased by the addition of a small amount of bicarbonate (2 mM). The additional presence of 1.1 mM acetazolamide results in a potential profile similar to that without bicarbonate. Potential changes also accompany the pH changes seen in the catalysed diffusion experiments of Jacobs and Stewart. Hypertonic additions of $NH_4Cl$ to red blood cells is accompanied by a large (> 35 mV) rapid depolarization. This potential, which slowly returns to an equilibrium value, can be rapidly dissipated with $NH_4HCO_3$ additions. The finding of electrical properties associated with proton disequilibria suggests that in experiments initiated by perturbation from equilibrium the role of all ions must be accounted for as well as indicating that competition between chloride and hydroxyl ions may play a significant role in controlling the electrical parameters of the membrane. (Supported by USPHS grants #AM 17433 and HL 09906.)

F-AM-E9 CATION TRANSPORT AND $^3$H-OUABAIN BINDING IN HUMAN RED CELLS LACKING THE RHESUS ANTIGENS ($Rh_{null}$-cells). P.K. Lauf and C.H. Joiner*, Department of Physiology & Pharmacology, Duke University Med. Ctr., Durham, N.C. 27710

The Rhesus (Rh) antigen complex (D,C,c,E,e) is one of the most important antigenic systems on human red cells and an integral lipoprotein of the cell membrane. Human red cells of type $Rh_{null}$ completely lack the Rh antigens and have an apparent membrane defect as evident by their shortened life span, increased fragility and tendency to hemolyze thus indicating a perturbation of cell volume regulation. We studied potassium ($K^+$)influx and $^3$H-ouabain binding in $Rh_{null}$-cells as compared to normal Rh antigen positive (Rh+)red cells The $Rh_{null}$-cells(patient B.R., through the courtesy of Dr.Seidl, Univ.Frankfurt W-Germany)were of high $K^+$ type and normal water content. However, in $Rh_{null}$-cells the ouabain-insensitive $K^+$-influx was 1.6-1.8 fold and the ouabain-sensitive $K^+$-pump influx 1.5 fold higher than in Rh+ cells. The $Na^+K^+$ATPase activity was 1.3 times higher in membranes from $Rh_{null}$-cells than in controls but without changes in the $K^+$-activation curve. Binding studies with $^3$H-ouabain revealed that $Rh_{null}$-cells bind about 1.4 times more ouabain(670 molecules/cell)than Rh+ cells(450-500 molecules/cell)while the rate of ouabain binding was unchanged. These findings are consistent with the hypothesis that the Rh antigen deficient human red cell studied has more $Na^+K^+$ pumps to control its volume. Since purified Rh antibody (anti-D) did not affect cation transport in Rh+ cells, the membrane defect in $Rh_{null}$-cells may be of pleiotropic nature (Supp.by PHS K4-GM 50,194 & 2 PO1-12,157).

F-AM-E10 CHLORIDE FLUXES AND VOLTAGE MEASUREMENTS IN SINGLE RED BLOOD CELLS. L.C. Stoner*, SUNY, Syracuse, N. Y. 13210 and F.M. Kregenow, NHLI, NIH, Bethesda, Md. 20014

Because $^{36}Cl$ penetrates the red cell membrane very rapidly, most studies of Cl transport have been conducted either at low temperatures or with indirect methods. We have developed a technique to measure directly the chloride flux and transmembrane voltage in single red blood cells from the salamander, Amphiuma. A single red cell is aspirated into a glass tube which has previously been lined with a silicone resin. Provided the inner diameter of the glass tube is 10-15 μ, the cell plugs the tube and separates the solution within the tube from that outside. Under these conditions the erythrocyte is cylindrical and has hemispherical-shaped ends exposed to each solution. Voltage is measured by using a Ling-Gerard microelectrode, positioned within the glass tube in such a way as to impale the entering erythrocyte. These intracellular potential measurements remain stable for a few seconds to a minute and average -8.0 mV (40 cells) at pH 6.5, -18.9 mV (49 cells) at pH 7.5, and -25.3 mV (19 cells) at pH 8.0. $^{36}Cl$ flux across the cell is measured by superfusing saline containing $^{36}Cl$ (introduced through a pipette within the glass tube) over one end of the cell and measuring the appearance of isotope in the bath at the other end. The rate at which $^{36}Cl$ appears in the bath can then be used to calculate Cl flux across the cell ($.843 \pm .05 \times 10^{-6}$ M cm$^2$/min). It is also possible to maintain a potential difference across the cell during the flux measurement by passing a small constant current. With a mean imposed potential of -230 mV (negative inside the glass tube), the flux increases less than 1-fold rather than the more than 100-fold one would theoretically expect if the flux were by simple diffusion through or around the cell. From this experiment and others, we conclude that the most of Cl passes through the cell membrane via an "electrically silent" mechanism.

F-AM-E11 COMPARATIVE STUDY OF ANION TRANSPORT IN DOG AND CAT RED CELLS. V. Castranova, M.J. Weise*, and J.F. Hoffman, Dept. of Physiology, Yale University School of Medicine, New Haven, Conn. 06510.

Self-exchange of chloride and sulfate in dog and cat red cells has been measured under equilibrium conditions at 0° and 37°C respectively. The rates of efflux for these anions in dog red cells ($^{o}k_{Cl}$ = 8.2 min$^{-1}$ and $^{o}k_{SO_4}$ = 1.9 hrs$^{-1}$) are approximately two-fold higher than those in cat cells ($^{o}k_{Cl}$ = 4.3 min$^{-1}$ and $^{o}k_{SO_4}$ = 1.0 hrs$^{-1}$). The Cl and $SO_4$ permeability of dog and cat red cells is affected by changes in cell volume, i.e., both shrinking and swelling these cells by approximately 10% leads to 10-20% increases in efflux. Cell volume was altered either by changing external osmolarity with sucrose or by placing cells with altered cation contents, prepared by preincubation in solutions of different tonicity and composition, in isotonic medium. Changing osmolarity by varying NaCl was not used to alter cell volume since Cl was found to inhibit $SO_4$ efflux to a similar extent in both dog and cat cells. Increasing $[SO_4]_{in}$ enhances $SO_4$ efflux to the same extent in both dog and cat cells, with neither species showing saturation of $SO_4$ transport with $[SO_4]_{in}$ up to 40 mM. Up to 96-97% of $SO_4$ efflux can be inhibited in dog and cat red cells by the amino reactive agent, SITS, with an apparent $K_I$ of 2.5 μM in both species. These data suggest that the anion transport systems in dog and cat red blood cells are very similar and imply that the lower permeability of cat red cells is due to a decrease in the number of transport sites rather than a lower affinity of the cat anion pathway for Cl or $SO_4$.

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F-AM-E12 TENSION FOR FAILURE IN FRAGMENTATION AND LYSIS OF HEREDITARY SPHEROCYTIC MEMBRANES. P. L. La Celle, P. T. La Celle* and B. D. Smith* Departments of Medicine and of Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, NY 14642.

The yield tension in shear of normal erythrocyte membranes is 2-8 x $10^{-2}$ dynes/cm, approximately three orders of magnitude less than isotropic tension at osmotic lysis. The hereditary spherocyte (HS), a pathologic stomatocytic/spherocytic erythrocyte is more susceptible to mechanical fragmentation and osmotic lysis than normal erythrocytes despite similar membrane protein and lipid components. The present study compares the characteristics of time-dependent local failure by fragmentation and isotropic tension at lysis of HS to normal erythrocyte membranes. Local failure was induced by stressing individual cell membranes in narrow (1-2μm) glass micropipettes and time ($t_F$) and tension recorded as indicators of local structural failure. Extension of membrane into larger (2.8μm) pipettes as a function of force was utilized to indicate elastic behavior. Isotropic tension at lysis was derived from sustained pressures required to cause lysis of osmotically swollen cells in 2.0μm micropipettes. The mean $t_F$ for HS membranes was 13.7 sec, 44% of that for normal membranes; tension at fragmentation was 4.1±0.4 x $10^{-2}$ dynes/cm and 2.9±0.3 for normal and HS. Tension for fragmentation was less than 80% that for relatively spherical senescent normal cells obtained by density separation. Elastic behavior of HS membranes was similar to normal over extension ratios up to 2.5. Mean isotropic tension for normal membrane lysis, 6.5±3.1 dyn/cm, exceeded that for HS, 4.6±3.2; and $10^2$ that for fragmentation. The significantly different failure characteristics of HS membranes suggest differences in molecular interactions important to structure.

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**F-AM-E13** EFFECTS OF CHOLERA ENTEROTOXIN ON CATION FLUXES, CELL VOLUME, AND CYCLIC AMP LEVELS IN THE TURKEY ERYTHROCYTE. Stephen A. Rudolph*, David E. Schafer, and Paul Greengard*, from the Departments of Pharmacology and Physiology, Yale University School of Medicine, New Haven, Conn. and Veterans Administration Hospital, West Haven, Conn.

Cholera enterotoxin (CE) has striking effects, believed to be mediated by cyclic AMP, on ion and water movement across intestinal and gallbladder epithelium. However, analysis of the mechanisms involved is complicated in these tissues by cellular heterogeneity and polarity. We have now found, in agreement with the results of Field (P.N.A.S., 71:3299-3303, 1974), that turkey erythrocytes incubated with CE show increased basal cyclic AMP levels and increased sensitivity of cyclic AMP levels to catecholamines. Treatment with CE also elevates sodium and potassium influx, increases the sensitivity of cation fluxes to catecholamines, and increases cell volume. Concomitant with this increase in cell volume, there is a progressive decrease in the maximal catecholamine effect on cation fluxes, which may be attributable to a net uptake of $K^+$, $Na^+$, and water which occurs in response to cyclic AMP at high extracellular $K^+$ concentrations; the accumulation of water and cations has a negative feedback effect on cation fluxes, rendering them insensitive to cyclic AMP. All the effects of CE on cation and water movements in the turkey erythrocyte appear to be accounted for by its effects on cyclic AMP accumulation, in the presence or absence of catecholamines. In contrast, although beta-adrenergic agonists and cyclic AMP stimulate the phosphorylation of a 240,000 MW plasma membrane protein (Rudolph, S.A., and P. Greengard, J. Biol. Chem., 249:5684-5687, 1974), it has not yet been possible to demonstrate a consistent effect of CE on the incorporation of $^{32}P$ into cellular phosphoproteins. (Supported by the USPHS and the Veterans Administration.)

**F-AM-E14** ANOMALOUS COAGGLUTINATION: CAN $Rh_o$ ANTIGEN TRANSLOCATE BETWEEN RED CELL MEMBRANES? B.J. Oberhardt, I.F. Miller*, and M.J. Lee, Technicon Science Center, Tarrytown, N.Y. 10591, and Univ. of Illinois, Chicago Circle 60680

Previously reported quantitative hemagglutination reactions (Fed. Proc. 33:1347(1974)) were subjected to further analysis. The reaction mixture consisted of antibody coated $Rh_o$+ human red blood cells and uncoated $Rh_o$+ or $Rh_o$- cells. Antibody bridge formation was facilitated by addition and subsequent neutralization of the cationic polyelectrolyte Polybrene$^R$, a "nonspecific" agglutinin which brings adjacent cell membranes into extremely close proximity. The reaction was performed in a continuous flow system and was monitored by means of optical density and radiolabeling techniques. In the present analysis a "phase diagram" was constructed consisting of agglutinated and free cell "phases", each phase containing antibody coated and uncoated cells. For initial reaction mixtures of $Rh_o$- cells only, no agglutinated phase was obtained. For initial reaction mixtures containing an excess of $Rh_o$- cells, in the presence of some antibody carrying $Rh_o$+ cells, a greater proportion of the $Rh_o$- cells were found in the agglutinated phase than would be expected. A passive steric inclusion process could not account for this result. The most reasonable explanation for this anomalous coagglutination appears to be the hypothesis that antigen or antigen-antibody complex can translocate from one red cell to another while the cell membranes are held in close proximity by the polyelectrolyte. This hypothesis, if true, may have important implications in cell interaction phenomena.

F-AM-F1 ELECTRICAL MICROSTIMULATION OF SMOOTH FIBERS. C. P. Bean, and R. J. King*†
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A theory of the microstimulation of individual myelinated nerve fibers has been given earlier.[1,2] We have extended this treatment to the case of a non-myelinated fiber. Under the assumption of a point electrode at a distance (d) from a line fiber with the requirement that the fiber to be excited must be locally depolarized by a critical potential difference ($V_c$) from its resting state, then a solution for the current threshold can be obtained in closed form. The fiber is characterized by a space constant ($\lambda$) and its surroundings by a homogeneous resistivity ($\rho$). The rheobase threshold current ($I_{th}$) is

$$I_{th} = -[4\pi\Delta V_c d/\rho]/[1 - (\pi d/\lambda)\,(H_o(d/\lambda) - Y_o(d/\lambda))]$$

where $H_o$ is the Struve function of zero order and $Y_o$ is the zero order Bessel function of the second kind. This expression has simple limiting values

$$I_{th} = -[4\pi\Delta V_c d/\rho] \text{ for } d<<\lambda, \quad I_{th} = -[4\pi\Delta V_c d^3/\rho\lambda^2] \text{ for } d>>\lambda$$

One consequence is that nearby stimulation tends not to distinguish among fibers while in the far-field larger fibers with correspondingly larger values of $\lambda$ are selectively stimulated.

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1. S. L. BeMent and J. B. Ranck, Jr., Exper. Neurol. 24, 171 (1969).
2. C. P. Bean in Appendix to C. Abzug et al., J. Physiol. (Lond.) 243, 499 (1974).

F-AM-F2 RANDOM WALK OF IONS IN MEMBRANE CHANNELS. P. Smejtek, Department of Physics, Portland State University, Portland, Oregon 97207

Monte Carlo computer model has been used to simulate the diffusion process in single channels. A channel is represented by a three dimensional network of jumping sites separated by symmetric barriers. The probability of jumps in the x,y and z directions is determined for each site from the applied voltage and from the ion-potential energy inside the channel. The ion-channel potential energy can be varied along the channel length as well as within the channel cross section. The model also allows for ion trapping on channel walls.

The model has been used to clarify some questions concerning the applicability of the Nernst-Planck equation, to obtain channel crossing probability distributions and other transport characteristics for channels of various size, potential energy profile and trap depth.

Supported by NIH grant ES 937.

F-AM-F3 MOLECULAR DYNAMIC (COMPUTER SIMULATION) CALCULATION OF THE EQUILIBRIUM AND KINETIC BEHAVIOR OF AN ION IN AN ION CHANNEL. G. Subramanian* and D.G. Levitt* (Intr. by E. Grim), Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

An exact solution for the behavior of an ion in a simplified model of an ion channel is obtained. The channel lies in the center of a helix which has a structure similar to that postulated for Gramicidin A. The channel (helix) atoms interact with each other through force constants appropriate for the atomic bonds of a polypeptide backbone and the ion interacts with each helix atom through electrostatic and Lennard-Jones forces. The ion and helix atoms are given an initial set of positions and velocities and the subsequent motion of the ion and helix atoms is then obtained from a computer solution to the Newtonian equations of motion. This provides, in effect, a moving picture of the ion and helix atoms. From the motion of the ion one can calculate, for example, the diffusion coefficient in the channel. In order to determine the potential energy profile in the channel, it is necessary to allow the channel to be distorted by the ion. We have obtained the potential profiles from the computer simulation by a self-consistent iteration procedure that allows for this distortion. It is an inherent assumption in the activation energy rate theory approach to ion transport that the ion thermally equilibrates after each jump. However, we have measured this rate of thermal equilibration and the results indicate that, if the helix is rigid enough, this rate is very slow and the rate theory approach cannot be used.

**F-AM-F4** ANALOGUE SOLUTION FOR ELECTRICAL CAPACITY OF MEMBRANE COVERED SQUARE CYLINDERS IN SQUARE ARRAY AT HIGH CONCENTRATION. K.S. Cole, Lab. of Biophysics, IRP, NINCDS, National Institutes of Health, Bethesda, Md. 20014

Analytical solutions of Laplace equations have given the electrical characteristics of membranes and interiors of spherical, ellipsoidal and cylindrical cells in suspensions and tissues from impedance measurements, but the underlying assumptions may be invalid above 50% volume concentrations. However resistance measurements on several non-conducting, close packing forms in two and three dimensions closely predicted volume concentrations up to 100% by equations derived from Maxwell and Rayleigh. Calculations of membrane capacities of cells in suspensions and tissues from extensions of theory, as developed by Fricke and by Cole, have been useful but of unknown validity at high concentrations. A resistor analogue has been used to solve the finite difference approximation to the Laplace equation for the parallel resistance and capacity of a square array of square cylindrical cells with surface capacity. An 11 x 11 array of resistors, simulating a quarter of the unit structure, was separated into intra- and extra-cellular regions by rows of capacitors corresponding to surface membrane areas from 3 x 3 to 11 x 11 or 7.5 to 100%. The extended Rayleigh equation predicted the cell concentrations and membrane capacities to within a few percent from boundary resistance and capacity measurements at low frequencies. This single example suggests that analytical solutions for other, similar two and three dimensional problems may be approximated up to near 100% concentrations and that there may be analytical justification for such analogue solutions of Laplace equations.

**F-AM-F5** A PHYSICAL MODEL OF NERVE AXON: IONIC DISTRIBUTION, SURFACE CHARGE AND RESTING POTENTIAL. D. C. Chang, Department of Pediatrics, Baylor College of Medicine and Department of Physics, Rice University, Houston, Texas 77005.

We have developed a theory of nerve excitation which is based on a set of assumptions different than those of the ionic theory of Hodgkin et. al. Here the axon is regarded as an open physical system which is free from any hypothetical mechanism such as membrane-situated "pump". The mobile ions are considered to be distributed at quasiequilibrium and the axoplasm is regarded as an analog of a cation exchanger. Following the spirit of the Debye-Hückel theory, some important features of the ionic distribution and electrical potential of the nerve fiber have been calculated. A new equation which describes the relationship between the resting potential and the ionic concentrations has also been derived. In comparison with published data and our recent experimental results, this equation appears to be in better agreement with the experimental observations than the classical equation of diffusion potential. (Supported by USPHS Grant GM-20154).

**F-AM-F6** EFFECT OF DIVALENT CATIONS ON THE SURFACE POTENTIAL OF CHARGED SURFACES: A DISCRETE CHARGE APPROACH. R. Sauve and S. Ohki, Department of Biophysical Sciences, State University of New York at Buffalo, Buffalo, NY 14226.

The surface potential produced by amphipatic molecules in contact with an electrolyte solution containing divalent cations was derived using a discrete charge approach. It was assumed that the system, amphipatic molecules-aqueous solution, could be divided into three regions; a non-polar region, an adsorption or polar region and an aqueous phase, each of them having a specific dielectric constant. A general expression for the electrical potential associated with an arbitrary arrangement of ionizable groups within the adsorption region was obtained in terms of Fourier-Bessel integrals. Numerical evaluation of local potentials for various bulk divalent cations concentrations in the case of an hexagonal arrangement of surface charges was performed and compared with results derived from a smeared surface charge approach. Possible application of this model to the problem of $Ca^{++}$ adsorption on negatively charged surfaces will be discussed.

F-AM-F7 ON THE ATOMIC ORIGIN OF CATION SCREENING AT CHARGED MEMBRANE SURFACES. J. D'Arrigo Department of Physiology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822.

There now exist numerous examples in the physiological literature of cation screening at the charged surfaces of both biological membranes and artificial bilayers. Past theoretical treatments of ionic screening (based on diffuse double layer theory) have involved useful but physically unrealistic assumptions regarding membrane, counter-ion, and/or water structure. This has resulted in the lack of a clear and realistic atomic representation of the ionic "screening" process. In an attempt to arrive at such an atomic picture, a model was devised having the following necessary and justifiable constraints: (1) The minimum internuclear distance between a negative site on the membrane and a cation screening the site is equal to the sum of the site's "equivalent" radius ($r_s$) + the diameter of a water molecule (~2.8 Å) + the crystal radius of the cation ($r_c$). (2) The average value for the dielectric constant (D*) over the internuclear distance (IND) is given by $D^* \approx 80(IND-r_s-r_c/IND)$. When this simple atomic model for ionic screening is employed in conjunction with equilibrium ion-selectivity theory, it is possible to quantitatively predict from Coulombic energy calculations the secondary stereospecific actions of certain alkaline-earth cations noted along with the predominant screening effect of these divalent cations at the surfaces of different types of membranes. The model also successfully predicts the transition from a predominantly screening situation to a predominantly binding situation observed with nerve (D'Arrigo, J. Physiol. 243:757, 1974) upon decreasing negative surface charge density. The accuracy of these predictions (1) depends most heavily upon the effective field strength (i.e., the "equivalent" radius) assigned to the individual membrane charge sites, and depends much less upon (2) the initial surface charge density or (3) whether a hexagonal or square array of charge sites is chosen. (Sup: NSF Grant BNS76-02647.)

F-AM-F8 ION EXCHANGE KINETICS IN AN ADSORBED PROTEIN FILM. M. Blank, W. Eisenberg* and J.S. Britten*. Department of Physiology, Columbia University, New York, N.Y. 10032

The stimulating and active ion fluxes in excitable membranes involve the entry of sodium or potassium ions into a medium where the other ionic species is normally present at a much higher concentration. If some of the cations originally present are bound to anionic sites on the membrane surface, ion exchange reactions may play a role in the process of excitation (Blank - J. Colloid Sci. 20:933, 1965). To study the properties of surface ion exchange reactions, a polarographic method was used. It was shown that Tl+ ions are bound to bovine serum albumin (BSA) at pH 8, and that the complex adsorbs rapidly at the mercury/water interface. When the polarity of the interface is reversed by an anodic pulse, $Hg^+$ ions are generated where the BSA-Tl complex is adsorbed, and the newly formed $Hg^+$ ions can exchange with the bound Tl+ ions. By pulsing the interface for very short times and then returning to cathodic polarizations, the concentrations of the two ionic species present can be determined by measuring the diffusion currents at -.3 volts, due to the $Hg^+$ ions only, and at -.9 volts, due to both $Hg^+$ and Tl+ ions. The data show that there is an additional current due to Tl+ ions that arises by an ion exchange process. The measurements also suggest that the ion fluxes and the ion concentration gradients driving these fluxes are much greater at shorter times.

F-AM-F9 IONIC ADSORPTION ON LIPIDS: BLOCKING BY TEA. F.F. Offner, Y.L. Chiu,* T. Krejske,* and S.H. Kim,* Technological Institute, Northwestern. University, Evanston, Il.60201

Competitive adsorption of ion species at the membrane interface plays an important role in the active membrane. Adsorption on a monolayer of phosphatidyl serine (bovine) was studied using a conventional Langmuir trough. Change in the interface potential was measured using the ionization method, at a constant surface pressure. $\Delta V$ for 0.1M $Ca^{++}$, $K^+$, and $Na^+$ were 400, 300, and 260 mV respectively, the lesser adsorption of $Na^+$ presumably being due to its more rigid hydration shell. Change with concentration followed an approximate Langmuir isotherm. Use of 0.01M TEA with 0.09M KCl reduces $\Delta V$ to 210 mV, the value found with 0.001M KCl. Thus TEA is very strongly adsorbed, sterically excluding other cations. The effect is similar to that found in electrochemistry: TEA binding is largely by induced dipole forces; the binding energy is thus quadratically related to the interface potential. This increases due to increased "IR drop" as the diffusion barrier shifts from the external to the internal interface on depolarization, and thus accounts for the progressive adsorption of TEA on depolarization of the axon. The adsorbed TEA sterically excludes other species from the membrane surface, and thus blocks the outflow of *any* ion species.

Supported by U.S.P.H.S. grant NS 08137. Mr. Krejske is now at the University of Wisconsin. Dr. Kim is now with Eastman Kodak Co.

**F-AM-F10** ORIGIN OF THE ELECTROCARDIOGRAM: INFLUENCE OF CELL GEOMETRY. L.V. Corbin II,* A.M. Scher, and A.C. Young, Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington 98195.

Several laboratories, including our own, have made quantitative attempts to predict electrocardiograms from real myocardial excitation data, assuming depolarization wavefronts to act as uniform electric dipole sheets. The results to date have been mediocre, even when accounting for boundaries and inhomogeneities. In stimulation experiments in dog ventricular tissue, we examined the assumption that myocardial depolarization wavefronts behave as uniform dipole sheets. Waves propagating transversely to fiber direction unexpectedly produced very different potentials than waves traveling longitudinally. The uniform dipole-sheet models predict that positive potentials should be seen ahead of the wave. Diverging waves approaching transversely to fiber direction, however, were actually preceded by zero or slightly negative potentials. Longitudinal waves, however, showed the traditional "approaching positivity," and receding waves of both types gave traditional negative potentials. This behavior can be explained with a model of excitation in parallel, long cylindrical cells with end-to-end and lateral contacts, in which the effective electric dipole moment of each partially depolarized cell points nearly along the cell axis. This model is in agreement with existing data concerning cardiac cellular morphology, cell-to-cell conduction, and activation velocities. Future forward ECG modeling, both qualitative and quantitative, will have to take cell orientation into account when converting depolarization wavefronts into equivalent electrical sources.

**F-AM-F11** DETECTION OF ELECTROMAGNETIC RADIATION FROM THE BRAIN. P. A. Anninos, S. Zenone* and C. Melas*. Department of Physics, Concordia University and Physics Department, Dawson College, Montreal, P. Q.

We are planning an experimental investigation of the Electromagnetic radiation (E.M.) from the brain based on our previously published model (Internat. Journal of Theor. Phys., Vol. 12, No. 1, 1975). Such investigation will consist in obtaining the usual form of the EEG from E.M. radiation detected in a region few centimeters away from the skull. Using a data acquisition system with a mini computer will permit the testing of detectors (of different characteristics) as many as 16 at a time for each trial. After we have satisfactory results and an optimum detector has been chosen, research will be carried out with the analysis of the EEG's from a particular individual. We expect the EEG so obtained to be more reliable than the EEG obtained by using electrodes in direct contact with the skull. In fact the steady polarizing currents always present in the latter case will be thus eliminated. This will provide a very useful diagnostic technique for clinical neurologists trying to determine the underlying neural processes responsible for mental illnesses.

**F-AM-G1** DETECTOR IMPROVEMENTS FOR CRYSTALLOGRAPHIC STRUCTURE ANALYSIS BY ELECTRON MICROSCOPY. R. M. Glaeser and I. A.-M. Kuo*, Division of Medical Physics and Donner Laboratory, University of California, Berkeley, California 94720

Structure analysis by electron microscopy is now possible at a resolution of better than 10 Å, using unfixed, unstained, hydrated biological specimens. For example, the three dimensional structure analysis of purple membrane at 7 Å resolution has recently been reported by Henderson and Unwin (Nature 257, 28 (1975)). Further advancement of this new experimental approach to biological structure determination requires the development of suitable data-recording and readout systems, which are capable of detecting single electron events in an image field of 10 cm diameter or more, with positional accuracy of 50 micrometers or better. A number of commercially available photographic plates have been tested under various conditions of photographic development. Kodak nuclear track emulsion NTB2 has shown the best characteristics, in terms of the requirements described above. For example, if the electron exposure on NTB2-coated plates is as low as $10^{-3}$electron/$\mu m^2$, then the optical density of the developed plate can be as high as 1.0 or 2.0 while the resolving power of the emulsion remains under 50 μm. Attainment of such a high optical density facilitates data readout on an automatic scanning densitometer, while at the same time the conditions of development used (Ultramicroscopy 1, 53 (1975)) are such that the fog level (i.e. detector noise) remains negligible. The use of NTB2-coated plates should enable data-recording at approximately 3.5 Å resolution, but further improvements in detector technology may be required at higher resolution.

**F-AM-G2** THE CHARACTERIZATION OF HETEROGENEOUS PROTEIN SYSTEMS BY LASER LIGHT SCATTERING TECHNIQUES: AN ANALYSIS OF TUBULIN PREPARATIONS.* J.S.Gethner‡† F.Gaskin,+ G.W.Flynn‡† and B.J.Berne‡† †Dept. of Chemistry, Columbia Univ., New York, N.Y. 10027 and +Depts. of Pathology & Biophysics, Albert Einstein Col. of Med., Bronx, N.Y. 10461

The composition of a heterogeneous solution has been investigated using Laser Light Scattering techniques. Methods for the analysis of light scattering data are extended from the case of a single component system to allow for the presence of multiple components. This technique affords several advantages over conventional methods of characterizing multicomponent systems. Experiments may be carried out on a solution at equilibrium in the absence of any external perturbations. Trace components of higher molecular weight may be easily detected. Because the light scattered from different components may be temporally resolved, it may be possible to observe changes in the relative concentrations of the components present as well as changes in the size distribution of each component.

Using these methods, a solution of tubulin prepared by the method of Shelanski, et.al.[1] was analyzed. These tubulin preparations assemble into microtubules at 37°C and disassemble into tubulin at 4°C. At 4°C a solution of tubulin centrifuged to remove protein greater than 20S is found to contain at least two components. The data is interpreted in terms of a heterogeneous mixture of tubulin (dimer,6S) and a higher molecular weight component having $D_{20,w} \leq 1.6 \times 10^{-7} cm^2/sec$. Carrying the tubulin through one cycle of temperature induced assembly/disassembly results in a sample with $D_{20,w} = 0.68 \times 10^{-7} cm^2/sec$. This indicates the presence of what is most likely a 30-36S "ring" structure of tubulin in addition to the high molecular weight component.

* Work supported by grants from the National Science Foun. & the U.S. Public Health Service

[1] M.L.Shelanski, F.Gaskin and C.Cantor, Proc. Nat. Acad. Sci. USA 70,765 (1973).

**F-AM-G3** TUBULIN ASSEMBLY PROMOTED BY DEAE DEXTRAN AND OTHER POLYCATIONS. H.P. Erickson, Department of Anatomy, Duke University Medical Center, Durham, N.C. 27710

In conditions normally used for the in vitro assembly of microtubules (MTs), (e.g., 0.05 M MES, 0.5 mM $MgCl_2$, 0.5 mM GTP, pH 6.5; 0.2 to 2.0 mg/ml tubulin), polycations are required for polymerization. The microtubule associated proteins (MAP), a heterogeneous group which binds to MTs during assembly and centrifugation from brain homogenates, will fulfill this requirement and the effect has been attributed to a MT-specific factor (tau) among the MAP. We have found, however, that several unrelated basic proteins, such as RNase A and histone, will substitute for the MAP in facilitating assembly of ion exchange purified tubulin. A non-protein polycation, DEAE dextran, average MW 2,000,000, was found to promote a small amount of assembly at concentrations as low as 0.001 mg/ml, and a substantial amount of assembly (30-70% of the tubulin, assayed by EM and pelleting) at 0.01 to 0.03 mg/ml (0.5 mg/ml tubulin throughout). The structures formed were almost entirely "double walled MTs", which have been determined to consist of a normal MT (inner wall) surrounded by a sheet of protofilaments (outer wall), which forms a spiral wrapping of 45° pitch. The subunit structure is thus virtually identical to that in the normal MT lattice. This assembly requires GTP and is inhibited by colchicine, calcium, and cold and is therefore considered to be essentially the same as normal MT assembly. It is suggested that a non-specific electrostatic interaction of the acidic tubulin molecules and the polycations is involved. A further experiment indicates that the polycations are involved in addition of each tubulin subunit to the MT lattice, and hence are required for elongation as well as for initiation of assembly: when intact MTs were run thru a phosphocellulose column at room temperature they were found to disassemble completely, presumably as the polycations (MAP) were bound to the column and removed from the MT ‡ tubulin mixture.

**F-AM-G4** THE ROLE OF TUBULIN-ASSOCIATED PROTEINS IN THE FORMATION OF TUBULIN RINGS. R.B. Vallee and G.G. Borisy*, Lab. of Molecular Biology, Univ. of Wisconsin, Madison, Wis.

Analytical ultracentrifugation and gel-filtration chromatography were used to investigate the structural relationship between a group of tubulin-associated proteins and tubulin rings, both of which have been implicated independently in playing a role in microtubule assembly (Borisy and Olmsted, Science 177, 1196, 1972; Murphy and Borisy, PNAS 72, 2696, 1975; Weingarten et al., PNAS 72, 1858, 1975). Microtubules were purified by 3 cycles of polymerization and depolymerization in 0.1 M PIPES, pH 6.94 containing 1.0 mM GTP. Separation of tubulin from its associated proteins was achieved with a DEAE-Sephadex A-50 column, the non-tubulin proteins coming through unbound (0.1 M PIPES, pH 6.94, 0.1 mM GTP and $MgSO_4$=PMG, plus 0.25 M NaCl), and tubulin eluting in a 0.5 M NaCl step. Purified tubulin in PMG buffer at 5°C showed a single 6S peak in the analytical ultracentrifuge. Addition of increasing amounts of the non-tubulin fraction resulted in the appearance first of a 20S and then a 30S peak, both of which appear to represent rings in the electron microscope. Unfractionated microtubule protein was found to elute as two major peaks after chromatography on Bio-Gel A-15m (pH 6.94-7.44, 5°), one at the position of purified tubulin, and the other near the void volume ($K_d$= 0.08-0.15). The latter peak contains rings, is capable of polymerization to form microtubules and contains several proteins of subunit molecular weight 270-345,000 at a relative concentration 2- to 3-fold greater than in the starting sample. These high molecular weight proteins show a different elution pattern when chromatographed in the absence of tubulin. The data indicate that non-tubulin factors are required for the formation of rings and that several proteins can be incorporated in the ring structure. The 20S and 30S species may represent rings differing in the content of high molecular weight proteins. (Supported by NSF grant GB-36454 to G.G.B. and NIH Postdoctoral fellowship CA01467 to R.B.V.)

**F-AM-G5** ISOLATION AND CHARACTERIZATION OF A FOLDED T4 DNA COMPLEX FROM T4 INFECTED ESCHERICHIA COLI. S.L. Hamilton and D.E. Pettijohn, Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado 80220

T4 DNA from Escherichia coli infected with phage T4 has been isolated in a condensed structure. The structure exhibits a narrow sedimentation profile and when isolated after 10 minutes of infection, it sediments at about 2500S. These isolated particles contain DNA, protein, and nascent RNA in a mass ratio of 1:1.3:1. The dimensions and DNA content of the structures have been determined using fluorescence microscopy and electron microscopy. They appear to contain the entire replicating pool of DNA of the infected cell. Their dimensions are also similar to that of the DNA pool observed in the infected cell. The proteins associated with the complex have been analyzed by slab gel electrophoresis. Only a very small fraction of the proteins of the host E.coli are bound to the DNA. Studies have been carried out to identify the elements responsible for the maintenance of the structure in a compact form. The effects of ethidium bromide on the condensed DNA suggest that its average superhelical density is much less than that of the isolated bacterial nucleoid, however, the results also suggest that there are domains in the DNA which become positively supercoiled in the presence of high concentrations of ethidium bromide. The existence of such domains would require that there be several constraints on the modes of rotation of the double-helical DNA in the condensed structure. In contrast to the bacterial nucleoid, the T4 DNA structure remains condensed after the RNA and protein components have been removed.

**F-AM-G6** A LEUKEMIA VIRUS STRUCTURE. S. Basu, K. F. Manly* and D. F. Parsons, Roswell Park Memorial Inst., Buffalo, N.Y. 14263

Moloney mouse leukemia virus has been harvested in continuous tissue culture and then purified by a sucrose density gradient method[1]. Both fixed and unfixed viruses have been subject to electron microscopic studies by the conventional negative staining, positive staining with uranyl acetate combined with critical point drying, critical point drying (Fr 113-13) followed by shadowcasting replication and, finally, by the wet replication technique[2]. A subpopulation of the virions are classically represented as tailed particles by negative staining. The positive staining technque combined with critical point drying method represents an overall circularity of the external envelope of these viruses with slight shrinkage. The nucleocapsids in most particles obtained after critical point drying are eccentric. In three dimensions, as obtained by shadowed replication of critical point dried viruses, the intact particles have polyhedral shapes; whereas in wet replicas of unfixed and unstained viruses they have spherical shape. No spikes or knobs of the external envelope have been found by any of the above techniques; however, these are being investigated using crude virus pellets to avoid any artifact due to high purification and also using cells with budding viruses.

Ref. 1. Manly, K. F. (1975). Analyt. Biochem. 63, 491-500.
2. Basu, S. and Parsons, D. F. J. Appl. Phys. (Part I & II) (in press).

F-AM-G7 COMPARISONS BETWEEN DIAMETERS MEASURED BY LASER BEAT FREQUENCY SPECTROSCOPY (LBFS) AND BY ELECTRON MICROSCOPY FOR MURINE MAMMARY TUMOR (MuMTV), ROUS SARCOMA (RSV), AND FELINE LEUKEMIA (FeLV) VIRUSES. I. Salmeen, L. Rimai (Research Staff, Ford Motor Co., Dearborn, Mich. 48121) R. B. Luftig* (Worcester Found. for Exper. Biol., Shrewsbury, Mass. 05145) L. Liebes, J. J. McCormick (Mich. Cancer Found., Detroit, Mich. 48201) Supported by NCI Contract NO1CP33226

The variance in the distribution of diameters of RNA tumor viruses measured in electron micrographs is 10-20% of the mean. LBFS of solutions with such particle size distributions would yield approximately single exponential correlation functions with time constants corresponding to light scattering intensity weighted average hydrodynamic diameters ($d_h$). (Koppel, J. Chem. Phys. 57 4814, 1972). If the electron micrographs (e.m.) represent solution size distributions, then $d_h$ should be compared not with the arithmetic mean (the usual way to report e.m. diameters), but with the light scattering intensity weighted average ($d_{iw}$) of the e.m. distribution. We measured $d_h$ for MuMTV, RSV, and FeLV on samples purified by density gradient centrifugation followed, after dialysis into phosphate buffer, by ultracentrifugation to clear particles with sedimentation coefficients greater than that of the singlet virions. Catalse crystal calibrated e.m. were obtained on FeLV both before and after and on RSV and MuMTV only before the final ultracentrifugation. We find that whereas the arithmetic mean of the e.m. size distributions are at least 10% smaller than $d_h$, the calculated $d_{iw}$ (using Mie theory to estimate relative scattering intensities) agree to within experimental error with $d_h$. $d_{iw}$ (nm) are: 147±3 (MuMTV), 139±3 (RSV), 161±5 (FeLV). $d_h$ (nm) are: 144±6 (MuMTV), 147±7 (RSV), 168±6(FeLV). This agreement suggests that the e.m. size distributions are representative of the size distributions in solution.

F-AM-G8 VIRAL SIZES, CONCENTRATIONS, AND ELECTROPHORETIC MOBILITIES BY THE NANOPAR ANALYZER. R. W. DeBlois, General Electric Research and Development Center, Schenectady, N.Y., 12301, and R. K. A. Wesley,* John L. Smith Memorial for Cancer Research, Pfizer, Inc., Maywood, N.J., 07607.

Viruses and other particles above 60 nm in diameter may be sized to a few nanometers, counted, and measured for mobilities to $1x10^{-6}cm^2/Vsec$ with the "nanopar" analyzer, based on the resistive pulse technique of the Coulter Counter and the use of nanometer-diameter pores made by the Nuclepore process. Size measurements against standard 109 nm latex spheres include T2 bacteriophage ($5.16\pm 0.15x10^{-16}cm^3$), Rauscher MuLV (122.2±1.4nm), Simian SV (109.7nm), M-PMV (140.0±2.5nm), RD114 (115±5nm), and Feline LV (134±5nm) in their natural hydrated states. Concentrations are measurable to about ±10% from $5x10^7$/ml either by latex sphere comparison or a hydrodynamic equation of flow. Electrophoretic measurements can yield absolute mobilities, reveal viral components, and in conjunction with size measurements may be used to characterize viruses. (Supported by NCI Contracts NO1-CP-3-3231 and NO1-CP-3-3234).

F-AM-G9 STRUCTURAL FEATURES OF FILAMENTOUS BACTERIAL VIRUSES FROM MEASUREMENTS OF MASS, LENGTH, COMPOSITION, AND UV SPECTRA. L.A. Day, S.A. Berkowitz,* and R.L. Wiseman. The Public Health Research Institute of the City of New York, New York, N.Y. (10016) and J. Newman* and H.L. Swinney* City College of the City University of New York, New York, N.Y. (10031).

A combination of techniques has been used to establish the average axial separation between subunits and neighboring nucleotides in fd virus (0.9 μ) and Pfl virus (2.1 μ), both of which contain circular single-stranded DNA encased in protein coats consisting of thousands of α-helical subunits of $5 \times 10^3$ daltons each. The ratio of nucleotides to subunits in Pfl is less than half that in fd, and in both cases this ratio is non-integral. The non-integral nucleotide/subunit ratios in these filamentous viruses is in contrast to the integral ratio of 3 for tobacco mosaic virus. The average axial nucleotide separation in fd is slightly more than 3 Å whereas in Pfl it is more than 6 Å. Pfl DNA, in situ, is in the most extended naturally occurring conformation known for any DNA. The axial protein subunit separation in fd is 1.4 times the subunit separation in Pfl. Thus the helical arrangements of subunits in these viruses differ greatly.

**F-AM-G10** ELECTRO-OPTICS OF T-BACTERIOPHAGES. Jan Greve*, Gert de Groot*, Paul Boontje* and Johan Blok* (Intr. by I. Tinoco, Jr.), Vrye Universiteit, de Boelelaan 1081, Amsterdam, The Netherlands.

Transient electric birefringence measurements were made of the bacteriophages $T_2$, $T_3$, $T_4$, $T_6$ and $T_7$. It appeared that the birefringence was negative for T-even phages and positive for $T_3$ and $T_7$. This may indicate that the DNA packing inside the head is different for the two classes of phages. Rotational diffusion coefficients ($D_{25,w}$) were determined as a function of pH, temperature and ionic strength. For $T_2$ and $T_6$ with retracted fibers $D_{25,w}$=333 $sec^{-1}$, whereas for $T_4$ $D_{25,w}$=280 $sec^{-1}$. Upon fiber extension these values decrease to 123 $sec^{-1}$, 133 $sec^{-1}$ and 159 $sec^{-1}$ for $T_2$, $T_4$ and $T_6$ respectively. For $T_4$ intermediate values of $D_{25,w}$ can be produced by a suitable choice of ionic conditions. This indicates that fiber unfolding occurs gradually and we favor a model in which the fibers unfan one after each other. A large increase in Kerr coefficient accompanies fiber unfolding for all T-even phages. For $T_2$ with unfolded fibers the Kerr coefficient does not depend on ionic strength, whereas for $T_6$ with extended fibers the Kerr coefficient decreases upon increase in ionic strength. Moreover, the Kerr coefficient of $T_6$ at low ionic strength is much greater than that of $T_2$ and $T_4$, both with unfolded and with retracted fibers. This indicates that $T_6$ has a charged site not present in $T_2$ and $T_4$. For $T_3$ $D_{25,w}$=4900 $sec^{-1}$ a value also found for $T_7$ at high ionic strengths. Unlike that found for $T_3$, both $D_{25,w}$ and Kerr coefficient of $T_7$ are variable, which indicates that a structural transition takes place for $T_7$ which does not occur for $T_3$.

**F-AM-G11** WET CHROMOSOME STRUCTURE. S. Basu, L. Pothier*, J. A. Huberman* and D. F. Parsons, Roswell Park Memorial Inst., Buffalo, N.Y. 14263

The wet replication technique[1,2] using a differentially pumped hydration chamber in a vacuum evaporator has been applied to metaphase chromosomes. Chinese hamster chromosomes were prepared by standard acid fixation and also, more recently, by isolation at a near neutral pH. The chromosomes were freed from cell debris by centrifugation and then deposited on hydrophilic (glow discharged) carbon coated grids with a Formvar support. The excess water on the grid was removed and the chromosomes were replicated at 20 Torr, corresponding to water vapor pressure at 22°. The sister chromatids of all acid-fixed chromosomes were fused and could not be distinguished, but a pattern of gross chromatid coiling could be observed. A loose wavy sheath of unknown material appeared to surround most acid-fixed chromosomes. This sheath is not observed when acid-fixed chromosomes are prepared by critical point drying using Freon 113-13 system. Chromosome fibers of about 500Å thickness could be resolved in chromosomes prepared by critical-point drying, but could not be seen using the wet replication technique, presumably due to the presence of the sheath. Details of chromosome structure are also being investigated by freeze-etch replication.

Refs. 1 & 2. S. Basu and D. F. Parsons, J. Appl. Physics (in press).

**F-AM-G12** THE MOLECULAR WEIGHT DEPENDENCE OF THE DIFFUSION COEFFICIENT OF CHROMATIN DETERMINED BY QUASIELASTIC LIGHT SCATTERING. Kenneth S. Schmitz, Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri and Barbara Ramsay-Shaw, Department of Chemistry, Duke University, Durham, North Carolina.

Chickens erythrocyte chromatin was digested with staphylococcal nuclease and separated by column chromatography. Various fractions were characterized according to their Svedberg constant, diffusion coefficient, optical properties, and the electrophoresis pattern of the extracted DNA. Several samples were checked at the end of the experimentation to determine the extent of degredation, with negative results. The molecular weights computed from the diffusion and sedimentation coefficients were in excellent agreement with the equilibrium sedimentation values at higher molecular weights, but differed by as much as 50% at low molecular weight. The data is summarized by the equation:

$$S_{20,w} = .0129M^{.57}$$

The .57 power dependence is indicative of a molecule with extensive hydrodynamic shielding.

**F-AM-G13** RECOVERY OF AMPHIPODS FROM DEEP OCEAN TRENCHES AT NEAR IN SITU PRESSURES: DESCRIPTION OF THE INSTRUMENT AND SCANNING ELECTRON MICROSCOPY OF ANIMAL-ASSOCIATED MICROBES. A. A. Yayanos, Scripps Institution of Oceanography, University of California, La Jolla, Calif. 92037

The ocean in its deepest parts is one of the most extreme environments found on earth. The nature of life at these depths which reach to 11,000 meters beneath the sea surface, is poorly understood with experiments being expensive and difficult. It has been well-recognized that the biology of the deep-sea will be greatly assisted by methods for the capture and maintenance of organisms at the conditions of their natural habitat. A device has been constructed and used to recover amphipods from one of the deepest parts of the Philippine Trench while maintaining the animals close to the high pressure of their deep-sea environment during recovery. The amphipods recovered belong to the family Lysianassidae and genus Hirondellea. The pressure retaining traps were deployed as free fall devices a total of 12 times. On two occasions no animals were recovered. On another occasion, over 1,000 animals were recovered with over 100 of them in the pressure retaining portion of the trap at a pressure of 858 atm. The pressure at the depth from which they were recovered is estimated at 981 atm. A long range goal is to trap amphipods and to study them in the laboratory. At the present, research is being done on the microbes which are associated with the amphipods. Cultures have been established and are being maintained at a pressure of 1,000 atm. Scanning electon microscopy has been used in the study of these microbes and some photographs will be shown. (Supported by NASA grant NGR 05-009-213.)

**F-AM-G14** NITROGEN ION ETCHING OF TMV AND T4 BACTERIOPHAGE. I. Bendet and N. Rizk*. Department of Biophysics and Microbiology, University of Pittsburgh, Pittsburgh, Pa. 15260.

The exposure of tobacco mosaic virus to a beam of nitrogen ions has been shown to remove portions of the nucleoprotein as a function of accelerating voltage and time of exposure to the beam (Rizk and Bendet, Ann. Proc. EMSA, 1972; Bendet and Rizk, Ann. Proc. EMSA, 1973). By following the change in the particle's diameter, the effect on repolymerized TMV protein has been found to be much greater than on the intact virus, suggesting that repolymerized protein is more susceptible than the nucleic acid to the ion etching. Experiments on T4 bacteriophage, in which relative head volumes were calculated from measured head dimensions assuming prolate ellipsoids of revolution, indicated that the effect of 5KV ions is greater than 2KV ions, and that an initial rapid decrease is followed by a more gradual decrease in head volume. Ion etching experiments carried out on bacteriophage containing either $^{35}S$ labeled protein or nucleic acid incorporating $^{3}H$-thymidine support the hypothesis that the portion of the head remaining is predominantly nucleic acid with the protein having been removed preferentially.

**F-AM-H1** THE INFLUENCE OF THE rev2-1 MUTATION ON UV-INDUCED REVERSION OF WELL DEFINED cyc1 ALLELES IN THE YEAST Saccharomyces cerevisiae. C.W. Lawrence and R. Christensen,* Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

The rev2-1 allele was isolated by Jeffrey Lemontt (Genetics 68: 21-33, 1971) as a mutation which decreased the UV-induced reversion of the ochre allele arg4-17. It was shown to have a somewhat smaller effect on the reversion of another ochre mutation lys1-1, but, unlike the similarly isolated rev1-1 and rev3-1 mutations, to have no effect on the UV-induced reversion of a presumed missense allele arg4-6 and little effect on forward mutation to auxotrophy (Lemontt, Molec. Gen. Genet. 119: 27-42, 1972). We have investigated the basis of the allele specific effect of the rev2-1 mutation by examining its influence on the UV-induced reversion of well defined cyc1 alleles, isolated and characterized by F. Sherman and J. Stewart by analysis of revertant proteins. Over all, the rev2-1 mutation was found to influence the reversion of cyc1 alleles to a smaller extent that arg4-17. The UV-induced reversion frequency of the ochre allele cyc1-9, which like that of arg4-17 is normally very high, was reduced by a factor of only about three, while arg4-17 reversion in these strains was reduced five to ten fold. Similar, though less well established, reductions were seen with some, but not all, other cyc1 nonsense alleles, though missense alleles appeared to be little affected. We conclude that the effect of the rev2-1 mutation is largely confined to the arg4-17 mutation alone.

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**F-AM-H2** KINETICS OF UV-INDUCED MUTAGENESIS IN YEAST. Friederike Eckardt* and R.H. Haynes, Department of Biology, York University, Toronto, Canada.

UV-induced mutation frequencies (mutants per survivor) in bacteria and yeast usually have been observed to increase quadratically with dose; normally these measurements are made over a limited dose range. We have measured the frequency of induced reversion to prototrophy ($ADE^+$ and $LYS^+$) in both wild-type and repair-deficient haploid strains of yeast (S. cerevisiae) over a relatively wide range of doses (1 to 1500 ergs/mm$^2$). Double log plots of these data (corrected for spontaneous revertants) show that at very low doses mutation induction is linear, rather than quadratic; the induction curve becomes quadratic at intermediate doses and at sufficiently high doses it becomes approximately exponential, approaching asymptotically a curve determined by the reciprocal of cell survival. In wild-type strains the transition from linear to quadratic kinetics occurs on the range of 50 to 70 ergs/mm$^2$ whereas in the excisionless rad 2-20 strain a similar transition occurs around 15 ergs/mm$^2$. Similar kinetics were observed for locus and suppressor mutations at both loci tested and so it is unlikely that this transition is associated with specific marker effects. Mutation frequencies in rad 2-20 are about 100 times greater than in wild type; this indicates that even though pre-mutational damage in yeast can be repaired by excision the cause of the transition must be sought elsewhere. Our results are consistent with the view that UV mutability in yeast is a single-event Poisson process, largely independent of cell killing; and that the transition from linear to quadratic induction kinetics arises from the interplay of mutation and inactivation processes in the intermediate dose region. (Supported by Deutsche Forschungsgemeinschaft and the National Research Council of Canada.)

**F-AM-H3** THE EFFECT OF ULTRAVIOLET IRRADIATION ON THE TEMPLATE ACTIVITY OF MDV-1, A RNA RELATED TO QB RNA. Frank Ryan and Milton P. Gordon, Dept. of Biochemistry, U. of Washington.

MDV-1 RNA is a small variant of QB RNA containing a know sequence of 218 nucleotides. The molecule is replicated by QB replicase. Two assays were used to determine the ultraviolet irradiation sensitivity of the RNA template activity. Both assays showed that the loss in template activity follows single hit kinetics for irradiation at 254 nm. A quantum yield of 2 X $10^{-3}$ was obtained in both assays. Uridine hydrates were found in the irradiated RNA. The products resulting from a replicase reaction using irradiated MDV-1 as template were studied. With small doses of irradiation (37% survival) the RNA templated reactions produced full length MDV-1 RNA plus four classes of additional RNA as determined by polyacrylamide gels. The additional classes of RNA were smaller than the MDV-1 template. With increasing doses less of the full-length MDV-1 and the larger two of the four additional classes were synthesized while the smaller two of the additional classes were synthesized to a greater extent. It was concluded that the irradiation lesion causes the replicase to stop at specific places on the RNA template.

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F-AM-H4 REPAIR OF DNA CONTAINING PSORALEN CROSS-LINKS IN TOLUENE-TREATED E. COLI, AND IN VIVO. G.H. Yoakum, R.S. Cole,* and R.R. Sinden, Department of Biochemistry, University of Georgia, Athens, Ga. 30602

Initial steps in the repair of cross-linked DNA involve removal of the covalent linkage between DNA strands. Biochemical and genetical characterization of this process in vivo indicates that uvrA, uvrB, uvrC, and polA (5'-3' exonuclease) gene products are required to complete the first and second cuts needed for cross-link removal. Digestion with specific DNAases', of DNA isolated from cells removing cross-links, shows that the first cut (uvrA&B endonuclease) is made on the 5' side of the cross-link producing a 3'-OH terminus. The second cut (polA exonuclease) is followed by the formation of a gap of approximately 1000 bases. Cross-link removal was carried out in toluene-treated E. coli, the rate is ca 50% of that measured in vivo and the reaction follows apparent first order kinetics. Since toluene-treated cells are permeable to small molecules, we have been able to determine the co-factor requirements for the removal of interstrand cross-links. The endonucleolytic step (1st cut) of cross-link removal requires co-factor concentrations of ATP (1.35 - 2 mM). In addition, uvrA, uvrB, and polAex strains incubated in 2 mM ATP do not remove cross-links. Strains deficient in the UV-endonuclease do not make the first cut in the cross-link removal reaction, whereas strains deficient in polA 5'-3' exonuclease complete the endonucleolytic step, but do not make the second cut required for completion of cross-link removal.

F-AM-H5 THE ATP DEPENDENCE OF THE INCISION AND RESYNTHESIS STEPS OF EXCISION REPAIR. W. E. Masker, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

Excision repair of ultraviolet (UV) radiation damage in Escherichia coli appears to be a relatively complicated process involving alternate pathways and a number of enzymes with similar in vitro activities. One approach to understanding the interplay of various enzymatic steps in repair pathways involves the use of a quasi in vitro system of permeabilized cells which allows easy introduction of chemical factors required for the incision, resynthesis, and ligation steps of excision repair. The UV repair process in one such system, using toluene-treated cells, has been well characterized and found to closely resemble in vivo excision repair. The present study makes use of this system to examine repair synthesis subsequent to incision. When polA strains of E. coli are made permeable, irradiated with UV, and incubated in a suitable assay mixture, UV-stimulated nonconservative DNA synthesis occurs only if ATP (or another triphosphate) is provided. Recently we have confirmed observations by other workers which show that the incision event is ATP-dependent. When UV-irradiated toluene-treated cells are incubated in the presence of ATP but without NAD or the four dNTPs, incision but no rejoining is observed. However, if these cells in which incision has already taken place are washed extensively to remove ATP, and then incubated again in complete assay mixture, nonconservative, UV-stimulated DNA synthesis occurs and is strongly stimulated by ATP. These data suggest that in addition to an ATP-dependent incision event there may be a second ATP-dependent step associated with repair replication in polA strains of E. coli. (Research supported by the U.S. Energy Research and Development Administration under contract with the Union Carbide Corporation.)

F-AM-H6 EXCISION REPAIR OF ULTRAVIOLET DAMAGE IN MUTANTS OF *ESCHERICHIA COLI* DEFICIENT IN DNA POLYMERASE I AND ITS 5'→3' EXONUCLEASE. Priscilla K. Cooper, Department of Biological Sciences, Stanford University, Stanford, California 94305.

*PolA1* mutants of *E. coli* deficient in DNA polymerase I are sensitive to ultraviolet (UV) and have been shown to perform increased repair synthesis compared to *pol*$^+$ strains (Cooper and Hanawalt, PNAS 69:1156 (1972)). We have interpreted this result as an increase in a large patch component of repair synthesis. Both polymerases II and III are also capable of performing repair replication and could account for this synthesis. *PolA1* mutants have nearly normal levels of the 5'→3' exonuclease and perform dimer excision at only slightly reduced rates. We have examined several mutants deficient in the 5'→3' exonuclease: The 5'→3' exonuclease deficient mutants *polAex1* and *polAex2* isolated by Konrad and Lehman, and *polA107* of Glickman, and the conditionally-lethal mutant BT4113ts of Olivera and Bonhoeffer, deficient in both polymerizing and exonuclease activity at the restrictive temperature. These strains all perform increased repair synthesis relative to that in a *pol*$^+$ strain. The UV sensitivity of the *ex1* and *ex2* mutants is intermediate between that of *polA1* and *pol*$^+$, and there is only a slight stimulation of UV-induced DNA degradation in these mutants, compared to extensive degradation in *polA1* and in BT4113ts. The enhancement of repair synthesis in the 5'→3' exonuclease-deficient mutants is less than in *polA1*, while the doubly deficient mutant BT4113ts shows even more repair synthesis than a *polA1* strain. We are examining the dimer excision capabilities of the various strains and have found that the *polAex2* mutant does excise dimers. We conclude that other enzymes can substitute for polymerase I in both the excision and resynthesis steps of excision-repair and that the resultant repair patches are of larger average size.

**F-AM-H7** DNA REPAIR MECHANISMS IN DROSOPHILA. J.B. Boyd* and R.B. Setlow, Department of Genetics, University of California, Davis, Ca. 95616; Biology Department, Brookhaven National Laboratory, Upton, N.Y. 11973.

Mutants of Drosophila melanogaster, which are sensitive to methyl methanesulfonate, were analyzed for their capacity to repair damage induced by x-rays and uv radiation. Analysis was performed on cell cultures derived from embryos of homozygous mutant stocks. The recombination-deficient mutant mei-9 (Baker and Carpenter, 1972, Genetics 71: 255-286) is deficient in the excision of pyrimidine dimers. Excision capacity was assayed with an endonuclease from M. luteus which is specific for pyrimidine dimers. Mutant and control cultures exhibit normal levels of photorepair. Postreplication repair following uv radiation has been analyzed in mutant stocks derived from a total of 11 complementation groups. Cultures were irradiated, pulse labeled, and incubated in the dark prior to analysis by alkaline sucrose gradient centrifugation. Kinetics of the molecular-weight increase in newly synthesized DNA were assayed after cells had been incubated in the presence or absence of caffeine. Mutants derived from 4 complementation groups have been tentatively assigned to two separate pathways of postreplication repair. One of the proposed pathways (RI, recombination independent) operates independently of recombination mechanisms and is caffeine insensitive. Recombination functions have been implicated in an alternate pathway (RD, recombination dependent) which is relatively sensitive to caffeine. All mutants investigated display a normal capacity to repair single strand breaks induced in DNA by x-rays. The data, in toto, suggest that Drosophila possess at least four DNA repair pathways: 1) photorepair, 2) excision repair, 3) postreplication repair-RD, and 4) postreplication repair-RI. (Supported by ERDA and the Guggenheim Foundation.)

**F-AM-H8** ENHANCEMENT OF THE RATE OF POST REPLICATION REPAIR IN MAMMALIAN CELLS. R. B. Setlow and Eleanor Grist* (Intr. by F. W. Studier), Biology Department, Brookhaven National Laboratory, Upton, New York 11973

DNA synthesized in vivo on damaged templates is initially smaller than that synthesized on undamaged ones. During subsequent incubation the small pieces are elongated and joined to give parental size molecules in a process called post replication repair (prr)--one part of which, in E. coli, is error-prone, inducible by UV[1], and associated with the appearance of a new protein[2]. We have looked for such a system in mammalian cells and have found that UV enhances the rate of prr in cells of Chinese hamster, normal humans, conventional Xeroderma pigmentosum (XP), and XP variants. Cells of the latter individuals are proficient in excision but are defective in prr[3] and they show larger UV stimulated enhancements (~2-fold) than the other human cells. The experimental procedure involved exposing cells to an inducing fluence of 0 or 2.5 $Jm^{-2}$ of 254 nm, incubating them for 2-18 h, and then exposing them to 0 or 5.0 $Jm^{-2}$ followed by a 0.5-1 h pulse of $^{3}H$ dThd, a chase of 0-1.5 h, and sedimentation in alkali. The amount of $^{3}H$ under the peak of parental DNA (labeled with $^{14}C$) measured prr. Our data support the idea that an induced, error-prone pathway is responsible for UV carcinogenesis. Work supported by the U. S. Energy Research and Development Administration.

1) S. G. Sedgwick, PNAS 72, 2753 (1975).
2) S. G. Sedgwick, Nature 255, 349 (1975).
3) A. R. Lehmann, et al., PNAS 72, 219 (1975).

**F-AM-H9** DEFECTIVE EXCISION REPAIR OF γ RAY-DAMAGED DNA IN HUMAN (ATAXIA TELANGIECTASIA) FIBROBLASTS. M.C. Paterson and B.P. Smith*, Biol. and Health Phys. Div., Atomic Energy of Canada Limited, Chalk River, Ontario K0J 1J0.

Humans afflicted with ataxia telangiectasia (AT), a rare neurologic disease eventuating in lymphoreticular malignancies, respond adversely to radiotherapy. This radiosensitivity is also observed at the cellular level, suggesting a defective DNA repair process as a root cause. We have thus assessed the ability of three AT diploid strains to execute two known γ repair processes: (i) rejoining of single-strand breaks, (ii) excision repair of damaged bases (detected as sites sensitive to endonuclease activity in a crude M. luteus extract). After delivering $Co^{60}$ γ-rays under anoxia to monolayer cultures, the fate of the radioproducts was followed by two complementary methods, in vitro enzymatic assay and isopycnic density centrifugation. AT and normal strains rejoined strand breaks with identical kinetics; however, sites disappeared in mutant strains at a markedly reduced rate. Concomitantly, the level of γ-induced repair replication in AT strains was appreciably less than in normal strains. Moreover, the mutant lines appeared otherwise normal, including their response to UV damage. It thus seems that the AT strains are specifically deficient in excision repair of γ-induced base damage, probably due to the presence of a defective γ endonuclease presumed to initiate the multistep process. The ramifications of our finding are many, such as (i) vividly demonstrating the importance of DNA repair processes in enabling man to overcome natural levels of ionizing radiation; and (ii) possibly implicating defective DNA repair in the etiology of neoplastic transformation.

**F-AM-H10** MODEL FOR IRREPARABLE CELL DAMAGE INDUCED BY IONIZING RADIATION OF DIFFERENT QUALITIES. Arthur Cole and Ratna Datta*, Physics Department, University of Texas System Cancer Center, Houston, Texas 77025.

A fraction of radiation induced cell damage appears to be irreparable. At low doses this damage is expressed as the initial slope of a log survival versus dose response and appears to follow single hit kinetics. We propose that a large amount of single hit damage results from energy deposits left by single particle traversals of single sensitive loci. This differs from the model for dual radiation action by Kellerer and Rossi which assumes that single hit damage results from the traversal of two separated sensitive loci by a single particle track. Results of studies with short range electron and alpha particle beams, which penetrate a short distance into the cell nucleus, favor a single track, single locus model which assumes that irreparable damage to a single sensitive locus occurs with the probability $p(\Delta E_i)$ where $\Delta E_i$ is the instantaneous energy deposited in the locus by the traversing particle. Using empirical and theoretical results, the frequency of occurrence of energy deposits $\Delta E_i$ in targets of various dimensions, t, was calculated for various radiation qualities and conditions. The total probability, which can be expressed as an inactivation cross section for particle beam studies, is the sum of all $p(\Delta E_i)$. Calculated responses correspond to observed survival responses for gamma ray and partly and fully penetrating electron and heavy ion beam irradiations for the conditions; t = 3nm and p = $4\Delta E^2$, $\Delta E \leq 0.5$ KeV or p = 1.0, $\Delta E \geq 0.5$ KeV. These results are consistent with the interpretation that massive energy deposits in a single duplex DNA molecule can produce irreversible cell damage.

**F-AM-H11** EFFECTS OF CIS-DICHLORODIAMMINEPLATINUM(II) ON GAMMA-IRRADIATED E. COLI. R.C. Richmond,* J.D. Zimbrick and D.L. Hykes,* Dept. of Radiation Biophysics, University of Kansas, Lawrence, KS 66045

The anti-tumor drug, cis-$PtCl_2(NH_3)_2$, has been tested on stationary phase suspensions of E. coli C 321 $thy^-$ for toxicity, radiation sensitization, and effect on thymine base-residue release from cellular DNA subsequent to irradiation. Toxicity for this drug is marginally established at $5 \times 10^{-5}$ M, and at this concentration sensitizes the bacteria to $^{60}Co$ gamma-radiation. Sensitization in $N_2$-saturated suspensions (enhancement ratio of 1.81) is about 30 percent greater than that obtained in $O_2$-saturated suspensions (enhancement ratio of 1.39). Additionally, a small component of low-dose radiation sensitivity is observed for bacteria irradiated in the presence of sensitizing concentrations of the Pt-complex under anoxic, but not oxic, conditions. No radiation sensitization by the Pt-complex is observed at $1 \times 10^{-5}$ M, a concentration that is non-toxic.

When stationary phase cells are obtained from growth medium containing tritiated thymine, and subsequently irradiated and treated under conditions designed to limit enzyme action, various tritiated products following irradiation are obtained from the cellular DNA. The product of highest yield has been identified as thymine, and more thymine is produced in the presence than absence of oxygen during irradiation. When cells are irradiated in the presence of a sensitizing concentration of cis-$PtCl_2(NH_3)_2$, the thymine yield is reduced and shows little variation between oxic and anoxic conditions. (Supported by NIH-NIGMS grant #GM-18927.)

**F-AM-H12** VARIATION IN TISSUE DISTRIBUTION OF $^{125}I$-LABELLED SUPEROXIDE DISMUTASE IN MICE. A. Petkau, K. Kelly*†, W. S. Chelack*, S. D. Pleskach*, and C. Barefoot*†, Medical Biophysics Branch, Whiteshell Nuclear Research Establishment, Atomic Energy of Canada Limited, Pinawa, Manitoba, Canada. †Immunology Department, University of Manitoba, Winnipeg, Manitoba, Canada.

The tyrosine residues of superoxide dismutase (SOD) from bovine erythrocytes were labelled with $^{125}I$ to a specific activity of $3.4 \times 10^6$ cpm/unit of enzyme. The labelled enzyme ($^{125}I$-SOD) was injected intravenously into mice and its tissue distribution examined. One-half to an hour after an injection, the $^{125}I$ activity in the blood, lungs, heart, and kidney began to decrease and approached background within 24 h. In the thymus, intestines, stomach, spleen, and liver, the maximum activity occurred 2 - 4 h after the injection and thereafter decreased rapidly. The specific activity of the radionuclide varied from one organ to another with the brain and kidneys showing the lowest and highest levels, respectively. The heterogeneous tissue distribution of $^{125}I$ activity was accompanied by larger variations in the $^{125}I$-SOD levels, as determined from elution profiles of tissue extracts on Bio-gel P60 columns that resolved free $^{125}I$ from $^{125}I$-SOD. The proportion bound to SOD varied from 5% in the stomach to 74% in the kidneys and was infinitesimal with respect to the endogenous levels of SOD in the tissues. From these data it is calculated that an intravenous dose of SOD, for which a dose modifying factor (DMF) of 1.15 has been obtained (Biochem. Biophys. Res. Commun. 65, 1975, 886-893), may raise the tissue levels by 0.08% in the liver to 3.8 and 30% in the thyroid and kidney, respectively. The variable turnover of the enzyme and its heterogeneous distribution may contribute towards making the radioprotective effect of SOD a multiplicative process involving a combination of random terms.

**F-AM-H13** PHOTOLABILITY OF DNA CONTAINING 5-BROMOURACIL: MECHANISMS OF λ-PHAGE INACTIVATION AND DOUBLE-STRAND BREAKAGE. R.R. Hewitt, K.A. Marburger,* E.M. Goldin, and P.M. Corry, Departments of Biology and Physics, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025

Hewitt and Marburger (Photochem. Photobiol. 21:413-417, 1975) determined that alkali-labile damage is produced by ultraviolet light (254 nm) with 4.2 times the frequency of single-strand breaks in DNA containing 5-bromouracil (5-BrUra) and no double-strand breaks were produced by fluences as high as 7.7 times the $D_o$ for single-strand breakage. Studies of the comparative inactivation of normal and 5-BrUra λ-phage on $uvr^+$ and $uvr^-$ *E. coli* hosts suggest that alkali-labile base damage is the lethal photoproduct in 5-BrUra λ-phage. The dose responses for double-strand breakage have been examined for 5-BrUra λ-DNA irradiated in two different buffers in which the frequencies of single-stranded breakage vary by about 4-fold. Double-strand breakage exhibited dose squared relationships with sensitivities that reflect the sensitivities for single-stranded breakage in each buffer. Present data support a conclusion that most double-strand breakage occurs by the accumulation of single-strand breaks produced at random in UV irradiated 5-BrUra λ-DNA.

**F-AM-H14** INDUCTION OF STABLE PROTEIN-DEOXYRIBONUCLEIC ACID ADDUCTS IN CHROMATIN BY ULTRAVIOLET LIGHT. G. F. Strniste, S. C. Rall*, and J. M. Hardin*, Cellular and Molecular Biology Group, Los Alamos Scientific Laboratory, U. of CA, Los Alamos, NM 87545, USA.

The formation of protein-DNA adducts in chromatin mediated by ultraviolet (uv) light was investigated in an attempt to compare alterations induced *in vitro* with those observed *in vivo*. Three methods of analysis were used to detect the formation of adducts in solutions of Chinese hamster cell chromatin exposed to uv light: 1) high salt membrane filter assay, 2) gel filtration and 3) isopycnic gradient analysis. The rate at which chromatin proteins are linked to DNA is constant with dose (methods 2,3) whereas the rate at which DNA is linked to protein diminishes with dose (methods 1,3). At a particular uv light dose there is an enhancement in adduct formation as the pH of the chromatin solution is lowered from 7.5 to 1.5 (methods 1,2,3). Furthermore, adjusting the ionic strength of the chromatin solution with NaCl enhances adduct formation up to 0.2 M. (methods 1,2). The addition of divalent metal ions to chromatin solutions prior to irradiation also enhances the crosslinking process (methods 1,2), maximum adduct formation at a particular uv light dose is observed at 2.5 mM, 5 mM or ≥ 10 mM for $Zn^{++}$, $Mn^{++}$, $Mg^{++}$, respectively. Analysis of adduct formation in chromatin preparations in which the chromatin proteins are labeled (radioisotopically) with different amino acid precursors (Lysine, Leucine, Tryptophan or combinations of these) indicate that the rate of linkage of non-histone chromatin proteins exceeds the rate of linkage of histone proteins. Qualitative analyses of isolated, DNase treated adduct proteins using gel electrophoresis and ion exchange chromatography are in progress. (This work was performed under the auspices of the U. S. Energy Research and Development Administration.)

**F-AM-H15** ABSENCE OF PHOTOENZYMATIC MONOMERIZATION OF PYRIMIDINE DIMERS IN NORMAL AND XERODERMA PIGMENTOSUM CELLS. J.E. Cleaver, M. Paterson, and E.C. Friedberg. Laboratory of Radiobiology, University of California, San Francisco, Calif., Chalk River Nuclear Laboratories, Ontario, Canada, and Department of Pathology, Stanford University Medical Center, California.

Photoenzymatic repair (PR), the monomerization of ultraviolet (UV) light induced pyrimidine dimers by illumination with visible light, has been considered a repair system absent from placental mammals, although present in marsupials and embryonic birds. We have re-evaluated this assumption by attempting to demonstrate PR in 3 normal and 7 xeroderma pigmentosum (XP) cell lines by irradiating with 13 to 130 $J/m^2$ of UV light under red safe light and illuminating with black, fluorescent or incandescent light sources for up to 18 hr. Pyrimidine dimers were quantitated with conventional chromatographic procedures or the UV endonuclease from *M. luteus*. Although our experiments did detect PR in chick fibroblasts, no evidence for PR could be found in any of the human cell lines, including one for which positive evidence for PR *in vivo* has previously been reported (Sutherland et al Proc. Natl. Acad. Sci. 72, 103, 1975). We conclude either that PR does not exist in human cells and the previous report represents an unexplained experimental result or that the procedures required for demonstrating PR *in vivo* in human cells require subtle factors that have not been explicitly identified at this time. Work supported by the U.S. Energy Research and Development Agency.

F-POS-G1 A MIXED-MODE SYSTEMS APPROACH TO SENSORY INTEGRATION AND LEARNING. Lawrence E. Mallach, Department of Biophysical Sciences, State University of New York at Buffalo, Amherst, NY 14226.

Mathematical techniques are developed for analyzing control systems which operate in dynamical and syntactical modes. Split brain, cortical lesion, and sensory deprivation studies support the dual mode description, as well as the necessity of integrating these modes of processing. The dualistic function of the hemispheres in human brain and the adaptive function have eluded mathematical description since mixed-mode systems do not fit into any conventional mathetmatical framework. Syntactical processing is rate-independent and therefore may be incommensurate with the real time of dynamical systems. The development of mathematical methods which can characterize the interaction of these two modes is essential to many areas of current interest. There is good evidence (Teuber, 1972) that frontal lobe function includes interaction of the dynamic and syntactic modes for analysis of current effector states with respect to anticipated results and integration of learned behaviors to produce new learned behavior. The temporal conjunction of the two modes forms a model which acts as a standard for comparison against on-going activity and constitutes a learned behavior when the syntactic mode is able to run in real time. It is suggested that the interaction of these two modes may be crucial for adaptive behavior and learning. Several examples of systems with mixed-mode behavior are studied, and their characteristics discussed.

F-POS-G2 EFFECT OF POSTNATAL STRESS ON MATURATION OF VISUAL EVOKED POTENTIAL. C. Torda. N.Y.Sch.OF PA.TRAINING,New York,N.Y.,10029(Mailing address:Fifth Ave & 100 Street,N.Y.,10029

Visual and auditory evoked potentials result from genetically predetermined processes, but are also influenced by environmental events, e.g. maturation and final shape of both the auditory and visual evoked potentials may change in animals reared under conditions of sensory deprivation. Exposure to recurrent postnatal stresses on maturation of visual and auditory evoked potentials were studied in the present work. Symptomfree neonates served as controls. Neonates subject to recurrent crying spells served as experimental subjects. Recurrent crying spells without any identifiable organic symptomatology qualified as manifestations of stressful experiences ( judged from their effect on steroid metabolism). In control neonates the visual $P_3$ wave became measurable during the second postnatal month. Different figures evoked differences in the shape of the $P_3$ wave by the end of the third postnatal month. In neonates undergoing recurrent crying spells visual $P_3$ wave became measurable only during the third month. Different figures evoked differences in the shape of the visual $P_3$ wave only by the end of the fourth month. Differences between controls and experimental subjects were statistically significant. Similar measurements of auditory evoked potentials revealed compensatory increase of the amplitude of the auditory $P_3$ wave. in the experimental subjects. According to current concepts maturation and behavior of the $P_3$ waves are intimately related to cognitive processes. The results suggest that exposure to postnatal stressful situations interfers with the normal development resulting in substitution of vision to hearing as principal way to perceive exogenous sensory experiences. This delay seems to result incollection of an excessive early memory bank of auditory engrams with unusual components, their reinforcement, and facilitation of recall.

F-POS-G3 BLOCKAGE OF IONIC CURRENTS BY YOHIMBINE IN SQUID GIANT AXON. R.J. Lipicky, D.L. Gilbert, and G. Ehrenstein, Dept. Pharmacology, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267; Lab. of Biophysics, NINCDS, National Institutes of Health, Bethesda, Md. 20014, and Marine Biological Laboratory, Woods Hole, Mass. 02543.

Yohimbine, an alkaloid, alpha adrenergic blocking agent, has been demonstrated to affect the action potential. We have studied the effects of yohimbine on the ionic currents in the squid giant axon by means of the voltage-clamp. Both sodium and potassium currents were reduced. The reduction of the sodium currents was such that the voltage at which the peak sodium current occurred was not changed by yohimbine, and the inactivation parameter, h, and its time constant were unchanged. Blockage of sodium channels occurred for both inward and outward sodium current and for both internally and externally applied yohimbine. The blockage was concentration-dependent in the concentration range of $1 \times 10^{-5}$ to $1 \times 10^{-4}$ gm/ml yohimbine. A depolarizing step pulsed once per second reduced the sodium current with each successive pulse, until a new steady state was reached. The new steady state and the rate at which the new steady state was achieved depended upon the frequency of pulsing. A detailed analysis of the voltage clamp data demonstrates that blockage of sodium channels by yohimbine depends explicitly upon membrane potential.

**F-POS-G4** SODIUM CONDUCTANCE KINETICS OF SQUID AXON MEMBRANES POISONED BY GRAYANOTOXIN I. I. Seyama* and T. Narahashi, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

We have previously reported that grayanotoxin I (GTX I) increases the resting sodium permeability of squid axon membranes thereby causing a large depolarization (Narahashi and Seyama, J. Physiol. 242, 471, 1974). GTX I has now been found to cause drastic changes in kinetics of sodium conductance. During a prolonged step depolarization of the axon internally exposed to GTX I (4-6 x $10^{-5}$M), a transient sodium current was followed by a slowly developing steady-state sodium current. The transient sodium current exhibited characteristics similar to those of normal axons except that the curves relating $m_\infty$, $\tau_m$, $h_\infty$, and $\tau_h$ to the membrane potential were all shifted in the direction of hyperpolarization along the potential axis. The slow sodium component was much smaller in magnitude than the transient component, and exhibited a negative conductance at a negative potential range. It was hardly inactivated during depolarization, and was less sensitive to tetrodotoxin than the transient component. It is concluded that GTX I causes (1) both m and h kinetics to shift in the hyperpolarizing direction, and (2) a slow, non-inactivating sodium component to appear. Implications of the present results to the observed increase in resting sodium permeability by GTX I are discussed. Supported by N.I.H. grant NS10823.

**F-POS-G5** CATION PERMEABILITY RATIOS OF NORMAL AND GRAYANOTOXIN-TREATED SQUID AXON MEMBRANES. T. Hironaka* and T. Narahashi (Intr. by C.H. Wu), Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

We have previously reported that resting squid axon membranes do not exhibit a high selectivity to various cations with a permeability ratio Na(1) : Li(0.83) : formamidine (1.34) : guanidine(1.49) : Cs(0.87) : methylguanidine(0.86) : methylamine(0.78). Grayanotoxin I (GTX I) depolarizes the membrane through a selective increase in sodium permeability, but does not drastically alter the permeability ratio (Hironaka and Narahashi, Fed. Proc. 34, 360, 1975). In order to characterize sodium channels, we have now measured the permeability ratios during peak current before and during internal perfusion 4 x $10^{-5}$ M GTX I. The reversal potentials for peak current were measured in the absence of potassium in the external medium in which test cations were substituted for sodium. The permeability ratios, calculated from the differences in the reversal potential between test cations and sodium, are Na(1) : Li(1.12) : formamidine(0.19) : guanidine(0.19) : Cs(0.085) : methylguanidine(0.061) : methylamine(0.036). GTX I did not markedly change the ratios. Thus sodium channels exhibit entirely different permeability ratios at rest and during activity indicating operationally different characteristics. Supported by N.I.H. grant NS10823.

**F-POS-G6** REMOVAL OF SODIUM CHANNEL INACTIVATION IN SQUID AXON MEMBRANES BY N-BROMOACETAMIDE. G.S. Oxford, C.H. Wu and T. Narahashi, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

N-bromoacetamide (NBA) and N-bromosuccinimide (NBS), selective cleaving reagents for tryptophanyl peptide bonds, were used to modify ionic channels in squid axon membranes. When applied externally both 1 mM NBA and NBS almost completely eliminate transient sodium current while exerting less effect upon potassium and leakage currents. Internal perfusion with NBA results in complete removal of normal sodium inactivation during 8 ms depolarizing voltage steps in a manner identical to the effects of internally perfused Pronase. Double pulse inactivation experiments demonstrate a component of Na inactivation which remains intact but is not accompanied by a decay of $I_{Na}$ during the prepulse at certain levels. Activation of $g_{Na}$ is unaffected by NBA for moderate depolarizations as $m_\infty$ of the Hodgkin-Huxley model is unchanged and $\tau_m$ only slightly increased. For small depolarizations the activation of $g_{Na}$ is better described by m than $m^3$. Kinetics of sodium tail currents upon repolarization to about -60 mV are unchanged by NBA, whereas a slow turn-on of $I_{Na}$ follows the tail at about -40 mV which kinetically matches the time course of activation at that potential. Our data indicate that (a) NBA may become a useful tool for studying Na channel activation and for characterizing the inactivation gating structure, (b) tryptophan may be involved in the lability of both the inactivation gate and Na ion pathway, (c) some degree of conditioned inactivation separate from normal Na inactivation remains after NBA treatment, and (d) in the absence of inactivation Na channels exhibit kinetic behavior consistent with dual mechanisms for opening (activation) and closing (tail kinetics). Supported by N.I.H. grant NS10823.

F-POS-G7 DYNAMICS OF AMINOPYRIDINE BLOCK OF POTASSIUM CHANNELS OF SQUID AXON MEMBRANE. J.Z. Yeh, G.S. Oxford, C.H. Wu and T. Narahashi, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

Aminopyridines (2-AP, 3-AP, and 4-AP) selectively block the K channel of squid axon membranes without affecting the Na channel in a manner dependent on the membrane potential, and the duration and frequency of pulse. They are effective either from external or internal membrane surface. K current rises more slowly in 3-AP and 4-AP than in 2-AP. This is presumably due to the progressive removal of aminopyridine molecules from the binding site rather than the slowing of n kinetics of the Hodgkin-Huxley model. In addition these compounds exhibit frequency-dependent block. In double pulse experiments, the dependency of recovery on prepulse duration suggests that the channel must be open to remove block. Twin-pulse experiments with varying pulse intervals reveal that re-establishment of the block after the first pulse is much slower in 3-AP and 4-AP than in 2-AP. Tonic K current block is greater in 3-AP and 4-AP than in 2-AP. These results indicate that 2-AP binds to and leaves from the binding site more quickly than 3-AP and 4-AP, which is manifest by differences in phasic and tonic components of block among the three species. In high K sea water, all of them block both inward and outward K currents, but to a much lesser extent than in normal sea water. Aminopyridines interact with the K channel in a manner different from tetraethylammonium, and will become useful tools to characterize the K channel and to eliminate the K current. Supported by N.I.H. grant NS10823.

F-POS-G8 EFFECTS OF THE AMINOPYRIDINES AND SPARTEINE ON MYXICOLA AXONS AND THE LOBSTER NEUROMUSCULAR JUNCTION. J. S. Colton, C. L. Schauf, and C. A. Colton, Departments of Physiology and Neurological Sciences, Rush Medical College, Chicago, Illinois, 60612.

The effects of the compounds 2-, 3-, and 4-aminopyridine (AP) and sparteine on membrane conductance changes were examined using both voltage-clamped Myxicola axons and the lobster neuromuscular junction. In Myxicola axons the aminopyridines very specifically inhibited the potassium conductance ($G_K$) at concentrations of 0.1 mM to 5 mM without any apparent effect on resting membrane potential or leak conductance. Concentrations of 2-AP in excess of 5 mM were needed to noticeably decrease the sodium conductance. Potassium conductance-voltage curves were variably shifted in the depolarized direction along the voltage axis with no significant change in shape. There were only minor changes in the kinetics of potassium activation. In high potassium solutions both inward and outward going potassium currents were equally sensitive to the aminopyridines. Sparteine was found to be a more potent, but less specific inhibitor of $G_K$. In contrast to the aminopyridines, sparteine was much more effective when applied to the axon at high pH, and in addition tended to produce a noticeable potassium inactivation. When applied to the neuromuscular junction, 2-AP and sparteine dramatically increased the amplitude of both excitatory and inhibitory post-junctional potentials, with little or no change being observed in resting potential, resting input conductance, reversal potential, or mEPP amplitude or frequency. Quantal content per fiber was increased by approximately a factor of three for excitatory synapses. (Supported by Research Career Development Award 1-K04-NS-00004-01 to C.L.S., National Multiple Sclerosis Society Grant RG-921-A1, and RPSLMC grant-in-aid 37120)

F-POS-G9 MEANS BY WHICH GYMNODINIUM BREVE RED TIDE TOXIN PRODUCES REPETITIVE FIRING IN SQUID AXONS. M. Westerfield*, Y. Kim*, G. Padilla and J.W. Moore, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N. C. 27710. (Intr. by F. Jöbsis).

A partially purified toxin(s) extracted from Gymnodinium breve red tide organisms elicits a spontaneous train of action potentials in the squid giant axon. These spikes are similar in form to the control but show an increase in the slope of recovery from the afterhyperpolarization. Voltage-clamp techniques revealed that the toxin has no effect on the normal sodium or potassium conductance changes produced by step depolarization. However, consistent with recovery following an action potential, GbTX speeds recovery of the "shut off" currents to their steady state values following a depolarization. With the resulting rapid recovery from the positive phase of the afterpotential in unclamped nerves, the membrane potential overshoots the resting potential, triggers another spike and, hence, produces repetitive firing. The most likely mechanism for this toxin-induced repetitive firing is an inward current which was found to be inhibited by prehyperpolarization or by application of TTX.

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**F-POS-G10** A CAPRICIOUS "BLOCKING" ION. CESIUM CAN INCREASE POTASSIUM CHANNEL CURRENTS. R.J. French*, and W.J. Adelman, Jr., Laboratory of Biophysics, IRP, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, Mass. 02543

Cesium ions have been previously shown to exert a strong blocking effect on currents through the potassium channel of squid giant axon. When added to the external medium, cesium blocks inward going currents, and when added to the internal perfusion fluid cesium blocks outward going currents. Both of these actions are strongly dependent on transmembrane voltage. We have found that external cesium also produces an increase in outward current when the axon is bathed in artificial seawater containing 240 mM potassium. Instantaneous conductances were determined from step changes in current that resulted from $\pm$ 10 mV steps in the membrane potential. These conductances were increased by 6.9 $\pm$ 2.3% (mean $\pm$ S.E. of mean) in 50 mM cesium, and 20.9 $\pm$ 2.8% in 200 mM cesium, over values seen in control solutions of the same ionic strength and potassium concentration. We saw no significant voltage dependence of the effect in the range of transmembrane potentials, E = 14 to 54 mV. A plausible qualitative explanation of the data is that a cesium ion lodges in the outer end of potassium channel and blocks the one-way influx through the channel without significantly changing the efflux. This would result in an increase in the net outward current as observed. The interpretation is consistent with small, but repeatable, negative shifts in the resting potential observed when cesium was added externally. It demands that, under the conditions of these experiments, there is a significant unidirectional backflux of ions through the potassium channels during passage of a net current across the membrane.

**F-POS-G11** DIFFERENTIAL SENSITIVITY OF APLYSIA NEURONS TO TEA: INTERACTION WITH $CA^{++}$ IONS. Hugh Bryant, Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024

Tetraethylammonium (TEA) ions are known to delay the restoration of the membrane potential following an action potential (AP) in a wide variety of neuronal and non-neuronal cells (e.g., frog peripheral nerve, squid giant axon, molluscan central neurons, paramecia, frog muscle fibers). In the abdominal ganglion of Aplysia, some neurons were shown to be particularly sensitive, and others relatively insensitive, to the presence of 50 mM TEA in the external bathing medium (Bryant and Weinreich, J. Physiol. 244:181-195). Those neurons relatively insensitive to extracellular TEA are found primarily in the left caudal quadrant of the ganglion (L7,L10-13) and have AP's which are rapidly blocked by the removal of $Na^+$ from the medium. Neurons sensitive to extracellular TEA (e.g., L1-L-6,R1-R4,R14,R15) are found in the other quadrants and are able to produce AP's in the absence of extracellular $Na^+$. Additionally, AP's in the giant cell (R2) axon, but not the soma, are blocked by $Na^+$ removal (Junge and Miller, Nature 252:155-156). Correspondingly, only the soma exhibits a distinctive sensitivity to TEA. It was further found that AP prolongation in normally TEA-sensitive somata (e.g., R2,L3) depended on the extracellular concentration of $Ca^{++}$: lowering $Ca^{++}$ markedly reduced, and raising it increased, AP duration in the presence of TEA. Extracellular $Mg^{++}$ had an opposite, but not nearly so dramatic, effect as $Ca^{++}$. These results suggest an active role of $Ca^{++}$ (and perhaps $Mg^{++}$) in membrane repolarization following AP initiation. (This work supported by USPHS fellowship to H.B. and NSF and NIH grants to Jose Segundo).

**F-POS-G12** RELEASE OF RADIO-N-ETHYLMALEIMIDE LABELED PROTEINS FROM SQUID GIANT AXON BY DEPOLARIZATION AND INTERNAL APPLICATION OF VARIOUS SALTS. H.C. Pant, I. Inoue*, I. Tasaki, and H. Gainer*. Lab of Neurobiology, NIMH and Behavioral Biology Branch, NICHD, NIH, Bethesda, Md. 20014.

Membrane proteins play an important role in the nerve excitation and molecular organization of an excitable cell. In the present studies attempts were made to elucidate the possible involvement of protein macromolecules in the excitability phenomena. Squid giant axons were perfused internally with an isotonic KF solution containing radioactive-N-ethylmaleimide after removing the axoplasm. The axon was depolarized by external application of a $K^+$-rich, $Ca^{++}$-containing solution. The perfusate was collected under such a depolarized state of the axon membrane and was compared with that collected in the repolarized state. The perfusate was analyzed for protein released from the axon interior by trichloroacetic acid precipitation and gel electrophoresis methods. It was found that during $K^+$-depolarization proteins were released from the nerve. When the external $K^+$-rich medium contains 4 mM EGTA and no $Ca^{++}$ ions, release of protein was suppressed. The effect of different internal anions on the protein release was also studied. The sequence of anions arranged in the order of decreasing effectiveness of protein release was found to be as follows: $SCN > Br > Cl \gg F$. The significance of these results will be discussed.

F-POS-G13 PROBABILITY OF A SPECIFIED RESPONSE IN STOCHASTICALLY MODIFIED "ALL OR NONE" SYSTEMS. M. Mangel and D. Ludwig,* Dept. of Mathematics, University of British Columbia, Vancouver, B.C., Canada

When stochastic factors are present, the behaviour of a system which exhibits "all or none" properties is no longer wholly determined by the initial conditions. A method has been developed for the approximate calculation of the probability of a specified response. The probability is conditioned on the initial data and is a solution of a diffusion equation. The method has been applied to the study of competition between species and has been compared with Monte Carlo experiments. The method can be used to describe threshold fluctuations in nerves. (It can be shown that the work of Lecar and Nossal (Bioph. J., 11: 1048, 1971) is a special case of this theory). Similar ideas can be used to calculate the behaviour of systems which contain unstable limit cycles.

F-POS-G14 ABOUT THE TOPOLOGY OF ELECTRICAL CIRCUITS AND THE MORPHOLOGY OF BIOLOGICAL SYSTEMS. R. Padrón* and T. Kirchhausen. Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, IVIC. Apartado 1827, Caracas 101, Venezuela.

A general method based in the calculation of the transfer function $Z(s)$ has been used for the determination of the electrical equivalent circuits of the barnacle muscle fiber and the isolated frog skin. This function was obtained in the frequency range 0.09Hz-0.55x10$^6$Hz from a Bode diagram of the modulus $|Z(f)|$ and phase $\theta(f)$ of the muscle and skin electrical input impedances. For these cases $Z(s)$ turns to be a quotient of finite polinomials of the form $Z(s) = (a_0s^2 + a_1s + a_2)/(b_0s^2 + b_1s + b_2)$; by using the canonical one-port theory (Voorman J.O., Philips Res. Repts.23:485-515(1968)) we have obtained the 12 electrical equivalent circuits with the minimum number of components (3 resistors and 2 capacitors). It is striking to note that eventhough these circuits are topologically different they are strictly equivalent from the electrical point of view. We conclude that although operationally the choice of a particular circuit topology may be useful, information about the morphological organization of the system can not be obtained.

F-POS-G15 REGULATION OF THRESHOLDS OF ADENYL CYCLASE RESPONSE. S. Brydon-Golz*, A. Bennun. Graduate School, Rutgers University, Newark, N.J. 07102

Calcium-treated membrane preparation of adenyl cyclase obtained from rat cerebral cortex showed activation by norepinephrine (NE) at pH 7.5 ± 0.3. At ATP concentrations below 1.0 mM, NE dependent activation never exceeded 2 fold. This indicated the desirability to study endogenous conditions that may restrict the activity of adenyl cyclase. The table shows that the higher ATP concentrations inhibit adenyl cyclase activity only at lower magnesium concentrations. On the other hand, ATPase activation shows no inhibition by excess ATP concentration over Mg concentration. Therefore, if the activity of the enzyme is restricted by Mg concentration, a mechanism for adenyl cyclase activation would become dependent on a decrease in ATP concentration. Thus, it is proposed that ATPase activity associated with nerve impulse transmission, by decreasing the ATP concentration of the membrane's microenvironment, may record an impulse as an increase in adenyl cyclase activity. A study of ATPase and adenyl cyclase thermodynamic stability in the membrane indicates that both enzymes show the same energy of activation dependence for their denaturation reaction.

| [ATP] (mM) | [Mg] (mM) | Adenyl Cyclase Act. mu moles cAMP/h/g |
|---|---|---|
| 0.4 | 0.1 | 0.0 |
| 1.0 | 0.1 | 0.0 |
| 2.5 | 0.1 | 0.0 |
| 0.1 | 0.4 | 28.8 |
| 0.4 | 0.4 | 14.5 |
| 1.0 | 0.4 | 0.0 |
| 2.5 | 0.4 | 0.0 |
| 0.1 | 1.0 | 48.8 |
| 0.4 | 1.0 | 43.2 |
| 1.0 | 1.0 | 27.1 |
| 2.5 | 1.0 | 0.0 |

**F-POS-G16** THE INFLUENCE OF PHOSPHODIESTERASE INHIBITORS ON THE K+ INDUCED DECREASE IN NAD(P)H LEVELS IN RAT BRAIN SLICES. E. Keller*, J.T. Cummins, Addiction Research Laboratory, V.A. Hospital, Sepulveda, Calif. 91343 - Dept. of Pharmacol., Univ. of Calif., Irvine.

Three PDE inhibitors, theophylline ($2 \times 10^{-3}$M), 3-isobutyl-1-methylxanthine ($10^{-4}$M), and 4-(3-butoxy-4-methyloxybenzyl)-2-imidazolidinone (RO 20-1724, $10^{-5}$M), were administered to rat cerebral cortex slices using a flow system in a Krebs Ringer media containing 0.75mM $Ca^{++}$. The changes in reduced cytochromes and pyridine nucleotide (NAD(P)H) levels were followed using dual wavelength spectrophotometric methods. The two xanthine derivatives increase the level of NAD(P)H over a period of three minutes. All three PDE inhibitors markedly potentiate the decrease in NAD(P)H levels produced by 30mM $K^+$. Similar results are obtained for cytochrome $a$-$a_3$. The dual effects of these inhibitors suggest at least two mechanisms in stimulating metabolic rates. The significant increase in NAD(P)H caused by addition of theophylline may be explained in terms of PDE inhibition which may lead to an increase in the rate of glycolysis. Our results, especially the enhancement by theophylline of the $K^+$ induced decrease in NAD(P)H and preliminary experiments with adenosine which indicate potentiation of the $K^+$ response, coupled with published cAMP data, suggest that regulatory systems other than accumulation of cAMP may play an important role in the regulation of $K^+$ stimulated brain metabolism. Theophylline is perhaps active in these other control systems through its influence on the pool(s) of adenosine phosphates. Data on the influence of theophylline on other modes of depolarization will be presented. It is possible that these studies may provide information helpful in understanding the mechanisms that more directly relate brain depolarization and metabolism.

F-POS-H1 ON THE INTERACTION OF LECITHINS BOTH WITH A PURIFIED HYDROPHOBIC PROTEIN FROM MYELIN AND WITH FATTY ACIDS AND LYSOLECITHIN. R. P. Rand, Brock University, St. Catharines, Ontario, D. Papahadjopoulos, Roswell Park, Buffalo and M. Moscarello*, The Hospital for Sick Children, Toronto.

A purified hydrophobic protein from human brain myelin was combined with egg lecithin, dipalmitoyl lecithin (DPL) or dimyristyl lecithin (DML) by drying a chloroform-methanol-water mixture of the components. Samples of protein/lipid weight ratio of approximately 40%/60% were investigated using X-ray diffraction and differential scanning calorimetry (DSC). Egg lecithin-protein mixtures added to various amounts of water form pure lamellar structures over the concentration range 90-60% dry weight. The lipid protein thickness in the lamellae varied from 40 Å to 35 Å over this range and was 1-2 Å thinner than that of pure egg lecithin. Lamellae thicknesses for DPL-portein or DML-protein mixtures were equal to that of the pure lipid alone, both above and below their transition temperatures. These results showed that the hydrophobic protein is mainly embedded within the lecithin bilayer thickness both above and below the lipid transition temperature. DSC and X-ray experiments showed that the lipid transition temperature of the lipid-protein lamellae was initially the same as that of the pure lipid but with time it broadened and increased compared to the pure lipid so that crystallized chains existed up to +14°C for egg lecithin and 56° for DPL. However, this increase was correlated with increased lysolecithin and fatty acid in the samples. Independent experiments indicated that both fatty acids and lysolecithin increase the transition temperature of lecithins.

F-POS-H2 INTERACTION OF APOPROTEIN FROM PORCINE HIGH-DENSITY LIPOPROTEIN WITH DIMYRISTOYL LECITHIN; THE STRUCTURE OF THE COMPLEXES. D. Atkinson*, H.M. Smith*, J. Dickson*, and J. Austin* (Intr. by G.G. Shipley), Biosciences Division, Unilever Research Laboratory Colworth/Welwyn, The Frythe, Welwyn, Herts., U.K.

The morphology and structural organisation of the complexes formed from the apoprotein of porcine high-density lipoprotein and dimyristoyl phosphatidylcholine (lecithin) have been studied using the technique of small-angle x-ray scattering. Scattering measurements made in solvents of varying electron density were interpreted in terms of a scattering-equivalent model for the structure of the complex. This model is described by an oblate ellipsoidal morphology with dimensions at 20°C: major axis 110 Å, minor axis 55 Å. Within this overall shape the lipid hydrocarbon chains are organised in an apolar core whilst the lipid polar head groups and protein are located in a outer shell 8.5 Å in thickness. The oblate morphology demonstrates that the structure of the complex is directed by the fundamental bilayer organisation of the lecithin. The dimension of the minor axis (55 Å) indicates that phospholipid hydrocarbon chains are orientated perpendicular to the interface.

F-POS-H3 A MODEL OF A MOLECULAR MECHANISM FOR OLFACTORY TRANSDUCTION. Gilbert Baumann, Department of Molecular Biology, Eastern Pennsylvania Psychiatric Institute, Philadelphia, PA 19129.

Olfactory transduction is the process by which the binding of an odorous molecule to the receptor membrane is translated into a change in the electrical activity of the receptor neuron. A molecular model of this process is proposed and tested in a model membrane system. Synthetic lipid bilayer membranes can be made electrically excitable by the addition of special molecules, such as certain antibiotics like alamethicin. Electrical excitation in model membranes modified in this way is caused by the voltage-controlled insertion of these molecules into the bilayer and their subsequent aggregation to form ion-conducting channels. The modifying compounds are referred to as channel-forming molecules. There is growing evidence that the action potential in excitable biological membranes functions in a similar way. Thermodynamically and kinetically the insertion and aggregation steps strongly depend upon the physico-chemical properties of the lipid bilayer. It is proposed that the odorous molecules in addition to binding to specific receptor proteins, as is generally hypothesized, also adsorb directly to the lipid bilayer. This process changes the bilayer properties and, in turn, modifies the electrical activity of the membrane. Initial studies have shown that no measurable electrical changes are caused by molecules which are odorous to humans in lipid bilayers without added channel-forming molecules. Excitable lipid bilayers displaying a rhythmic action potential, however, can respond to small concentrations of such odorous molecules and in some cases block the rhythmic firing very abruptly. In this model odor specificity could arise from the chemical composition of the receptor membrane and the type of channel-forming molecule present.

**F-POS-H4** A STRUCTURAL DIFFERENCE BETWEEN PHOSPHOLIPID DISPERSIONS AND VESICLES AS REVEALED BY RAMAN SPECTROSCOPY. Bruce P. Gaber and Warner L. Peticolas, Department of Chemistry, University of Oregon, Eugene, Ore. 97403.

It has been recently suggested that differences might exist between the molecular structure of large multilamellar phosphospholipid dispersions and small (radius 125 Å) single-bilayered vesicles. The proposed structural difference is postulated to arise from disruption of orderly hydrocarbon chain packing induced by the small radius of curvature of the vesicles. Consequently vesicles would exhibit a less-ordered hydrocarbon interior than would a dispersion of the same phospholipid. To test this hypothesis we have examined the structure-sensitive C-C stretching and C-H stretching regions of the Raman spectra of dipalmitoyl phosphotidyl choline (DPPC), dimyristoyl phosphatidyl choline (DMPC) and egg phosphotidyl choline (EPC) prepared both as dispersions (mechanically homogenized above Tc) and vesicles (sonicated and then sized on Sepharose 4-B). Spectra of DPPC dispersions below Tc reveal a chain order intermediate between that of solid palmitic acid and DPPC dissolved in $CHCl_3$. Vesicles of DPPC are found to be consistently more disordered than the dispersions. These results may be roughly quantitated if the data are normalized in terms of an order parameter S which compares them to disordered chains (S=0) and highly ordered chains (S=1). Expressed in these terms, DPPC dispersions have S=0.48 while for DPPC vesicles S=0.34. These differences become negligible at Tc where for dispersions S=0.18 and for vesicles S=0.21. The actual Tc for DPPC vesicles, as determined by Raman spectroscopy, appears to be slightly lower (~2°C) than for DPPC dispersions. Similar results were observed with DMPC. When the Raman spectra of dispersions and vesicles of EPC are compared, they are found to be virtually indistinguishable. (Supported by Research Corp. (to B.P.G.) and NSF BMS-05468-A01 (to W.L.P.)).

**F-POS-H5** PHYSICAL CHEMISTRY OF PLANAR BILAYER MEMBRANES. Stephen H. White, Department of Physiology, University of California, Irvine, California, 92717.

Measurements of the specific geometric capacitance ($C_g$) of planar bilayers as a function temperature (T) have been made under equilibrium conditions. For bilayers formed from glycerol-1-monoolein in n-hexadecane, $C_g$ decreases linearly from $0.6291\pm0.0020$(SE) $\mu F/cm^2$ (T=20°C) to $0.5632\pm0.0024$(SE) $\mu F/cm^2$ (T=35°C). The n-hexadecane is distributed between microlenses and regions of true bilayer. The amount of solvent in the bilayer determines in part the bilayer thickness and consequently $C_g$. If the structure of the bilayer is known, then the mole fraction ($X_s$) of solvent in the bilayer as a function of temperature can be calculated from the $C_g(T)$ data. Several different methods of estimating $X_s(T)$ have been tried and the enthalpies ($\Delta H$) and entropies ($\Delta S$) of transfer of hexadecane from microlenses to bilayer estimated. $\Delta H$ and $\Delta S$ are always positive and much larger than expected for simple mixtures of alkanes in alkenes. For example, treating the bilayer as an "ideal" two dimensional liquid (F.M. Fowkes, J. Phys. Chem. 66 (1962), 1863) leads to $\Delta H=9.03\pm0.39$kcal/mole and $\Delta S=30.1\pm1.3$ eu. It is interesting to compare these numbers with those calculated for the transition of 1 mole of a $C_{15}$ hydrocarbon from bulk liquid to gas: $\Delta H_{trans}=21.6$kcal and $\Delta S_{trans}=32.6$ eu (N.L. Gershfeld and R.E. Pagano, J. Phys. Chem. 76 (1972), 1231).

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**F-POS-H6** THERMAL TRANSITIONS AND STRUCTURAL PROPERTIES OF CHOLESTANYL ESTERS AND BINARY MIXTURES OF CHOLESTANYL AND CHOLESTERYL ESTERS. B. E. North, G. G. Shipley, and D. M. Small, Biophysics Division, Boston University School of Medicine, Boston, Mass. 02118

5α-Cholestan-3β-yl esters of ten saturated and 3 unsaturated aliphatic acids have been examined by differential scanning calorimetry, polarizing microscopy, and x-ray powder diffraction. Transition temperatures, enthalpies, and entropies are compared with analogous cholesterol esters. Crystalline cholestanyl esters of even numbered n-alkanoic acids from $C_2$ to $C_{20}$ melt to isotropic liquids. The $C_8$ to $C_{20}$ esters have progressively increasing crystalline melting temperatures from 76 to 99°C, linearly increasing entropies from 28 to 61 cal $deg^{-1}$ $mole^{-1}$, and possess similar x-ray diffraction patterns, suggesting that these compounds form an isostructural series. In contrast, the analogous cholesteryl esters exhibit polymorphism. X-ray diffraction evidence and the fact that cholestanyl myristate-cholesteryl myristate mixtures form solid solutions, with transition temperatures which change progressively from that of one component to that of the other, provide evidence that the two myristate esters are isostructural. The cholestanyl myristate-cholesteryl oleate binary system was also examined and exhibits a eutectic containing 5% cholestanyl myristate. Crystalline cholestanyl oleate melts to an isotropic liquid at 29°C(cf. cholesteryl oleate 51°), whereas cholestanyl linoleate and linolenate, unlike the analogous cholesteryl esters, fail to crystallize, even after several months at -20°C. Cholestanyl esters of even numbered saturated acids from $C_4$ to $C_{14}$ form monotropic cholesteric mesophases. Esters $C_{10}$, $C_{12}$, and $C_{14}$ also form smectic mesophases. The smectic to cholesteric and cholesteric to isotropic transition temperatures of the cholestanyl esters are lower than corresponding temperatures of cholesteryl ester analogues, suggesting that the Δ5 double bond in cholesterol increases the thermal stability of the mesophases of cholesteryl esters.

F-POS-H7 A SPIN LABEL TECHNIQUE FOR MONITORING THE CHARGE DENSITY OF BIOMEMBRANES.
R.J. Mehlhorn and L. Packer. Membrane Bioenergetics Group, Energy and Environment Division, Lawrence Berkeley Laboratory, University of California, Berkeley, Calif. 94720

The spin labeled cationic detergent 4-(dodecyl dimethyl ammonium)-1-oxyl-2,2,6,6-tetramethyl piperidine bromide, exhibits two signals in an aqueous membrane suspension, e.g., phospholipid vesicles, mitochondria or red cells, corresponding to a partitioning of the spin label between aqueous and membrane domains. The degree of partitioning displays a marked sensitivity to changes in the charge density on the membrane as shown by treatment of egg lecithin vesicles with small concentrations of cationic and anionic detergents.

In other experiments, the uncharged spin label 2,2-dimethyl-5,5-dibutyl-N-oxazolidinyloxyl which also exhibits a partitioned signal arising from aqueous and membrane domains showed no detectable effects when egg lecithin vesicles were treated with detergent levels used to alter the vesicle charge density.

The combination of both spin labels can be used to estimate the charge density on a membrane with a sensitivity corresponding to about 1 charge equivalent/100 phospholipids.
(Supported by ERDA)

F-POS-H8 A NEW TYPE OF ELECTROSTATIC BOUNDARY POTENTIAL IN LIPID BILAYERS.
O. Andersen (Dept. Physiology & Biophysics, Cornell University Medical College); S. Feldberg*, H. Nakadomari* (Division of Molecular Science, Dept. Applied Science, Brookhaven National Laboratory); S. Levy*, S. McLaughlin (Dept. Physiology & Biophysics, SUNY Stony Brook)

It is well established that surface potentials on lipid bilayers can arise from either charges or molecular dipoles located at the membrane solution interface. If a membrane is exposed to a lipid soluble ion such as tetraphenyl borate or dipicrylamine, however, these ions will adsorb at a plane in the interior of the membrane a few Angstroms from the interface and will produce a change in the electrostatic potential within the membrane which may be described by a three layer capacitor model. Two identical outer layers represent the region between the plane of the adsorbed ions and the plane of the aqueous counterions. A middle layer represents the region between the planes of the adsorbed ions. The model equations predict the change in boundary potential produced across the outer regions and the associated modification of the adsorption equilibrium. The theory provides a self consistent interpretation of voltage-clamp, charge-pulse and "probe" studies of the adsorption of these anions into lipid bilayers. The high local concentration of gating charges in the vicinity of sodium channels in excitable membranes suggests that the existence of boundary potentials has important implications for a proper analysis of the movement of these charges in excitable membranes.

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F-POS-H9 IMAGES OF MOLECULAR FLIP-FLOP IN ASYMMETRIC LIPID BILAYERS.
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Physiologically important divalent cations have been electron microscopically visualized as the headgroups of oriented fatty acid bilayers in the absence of fixatives, stains or embeddments. Langmuir-Blodgett multilayers were built-up in a chemically coded sequence upon an epoxy wafer from which thin (~500Å) transverse sections were cut for direct EM examination. Decoding of the resultant intrinsic images has established that the apparent density of the alkali earth headgroups decreases with their atomic number in the order Ba>Sr>Ca>Mg, with unsaponified carboxyl headgroups being invisible. Bilayer absolute electron density profiles have been computed from x-ray diffraction studies of the same specimens. The diffraction results have verified the microscopically observed relationships and have established that the EM detectability threshold of these headgroups lies near 0.6 $e^-/A^3$ when about 60 molecules are superimposed in thin sections.

Asymmetric bilayers have been constructed with visible barium headgroups on one face and invisible carboxyl groups on the other. These Ba/COOH bilayers present intrinsic images with a primary lamellar spacing which is double that of control Ba/Ba symmetric bilayers as predicted by the formation process. The asymmetric bilayers also exhibit secondary fine dense lines which occur in a stepwise manner along the bilayer. The secondary lines appear at integral spacings of single bilayers and are uninfluenced by the inclusion of EDTA in the subsolution of the parent fatty acid monolayer. The bilayer electron density transposition has been confirmed by x-ray diffraction analysis. The results are interpreted in the context of X-Y multilayer inversions in which intact blocks of the bilayer are thought to flip over during formation.

**F-POS-H10** POSITRON ANNIHILATION AS A PROBE OF STRUCTURE OF BOUND WATER. R.H. Stinson, J. Fabian*, E.A. Williams* and I.K. MacKenzie*, Department of Physics, University of Guelph, Guelph, Ontario, Canada.

Positrons annihilate with electrons in bulk water with lifetimes and momentum distributions which are different from those observed in ice. We have carried out such studies in water, lamellar lecithin and model membrane systems (80% lecithin and 20% water). Results obtained using NMR and other techniques suggest that all the water molecules are tightly bound to the polar head groups at this concentration. Above 20°C we find that the mixture gives results which are apparently a simple addition of contributions from lecithin and bulk water taken separately. However, below 20°C there is a significant change in the results from the mixture, one possible explanation of which is that the water has undergone a change of structure towards a more ice-like state. Momentum distributions in annihilation will also be discussed.

**F-POS-H11** CALORIMETRIC STUDIES OF THE THERMOTROPIC BEHAVIOR OF AQUEOUS DISPERSIONS OF NATURAL AND SYNTHETIC SPHINGOMYELINS. Y. Barenholz*, J. Suurkuusk*, D. Mountcastle, T.E. Thompson and R.L. Biltonen*. Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia, 22901.

A recently developed differential scanning calorimeter has been used to characterize the thermotropic behavior of aqueous dispersions of liposomes containing sphingomyelin. Liposomes derived from sheep brain sphingomyelin exhibit a broad gel-liquid crystalline phase transition in the temperature range of 20-45°C. The transition is characterized by maxima in the heat capacity function at 31.2° and 37.1° and a total enthalpy change of 7.2±0.4 kcal/mole. Beef brain sphingomyelin liposomes behave similarly but exhibit heat capacity maxima at 30°, 32°, and 37° and a total enthalpy change of 7.4 kcal/mole. The thermotropic behavior of four pure synthetic sphingomyelins ($C_{16:0}$, $C_{18:0}$ and $C_{24:0}$ sphingomyelin and $C_{16:0}$ dihydrosphingomyelin) is reminiscent of that of multilamellar lecithin liposomes in that a single, sharp main transition is observed. Results obtained for liposomes containing mixtures of different sphingomyelins are more complex. These results indicate that the thermotropic behavior of sphingomyelin-containing liposomes is a complex function of the exact composition and that the behavior of liposomes derived from natural sphingomyelins cannot be explained in terms of phase separation of the individual components.

**F-POS-H12** A DIFFERENTIAL SCANNING CALORIMETER BASED ON THE HEAT-LEAK PRINCIPLE. J. Suurkuusk*, D.M. Mountcastle*, and R.L. Biltonen*. (Intr. by D. Kupke) Department of Biochemistry, University of Virginia, Charlottesville, Virginia

A precise and accurate differential scanning calorimeter based on the original design of Ross and Goldberg (Thermochimica Acta 10, 143 (1974)) has been developed. The calorimeter consists of two cells, each sandwiched between a pair of commercial thermoelectric modules in contact with a copper heat sink. The modules of the cells are connected in series with opposing polarity so when the cell temperatures are equal, the differential voltage is zero. The heat sink is positioned inside an adiabatic shield insulated from the surroundings. When the temperature is increased at constant rate, temperature differences are created between the heat sink and cells. The measured voltage is proportional to the temperature difference, and thus to the heat capacity difference, between cells. Due to imperfectly matched thermoelectrical module pairs and a temperature dependence of the calorimetric constants, an extensive calculation is necessary to obtain $\Delta C$ as a function of temperature. Improved matching of the thermoelectric modules and attainment of a more constant scanning rate will reduce the complexity of the calculation. The characteristics of the current version of this calorimeter are: 1) Scanning rate: 3-50°/hr 2) Temperature range: 0-100°C 3) Precision: ±25μcal/deg. 4) Absolute accuracy: better than ±100μcal/deg. 5) Sample volume: 0.7ml. Thus far this calorimeter has been successfully applied to thermodynamic studies of dilute solutions of nucleic acids and phospholipid dispersions.

**F-POS-H13** A CALORIMETRIC AND FLUORESCENT PROBE STUDY OF THE GEL-LIQUID CRYSTALLINE PHASE TRANSITION IN SMALL, SINGLE-LAMELLAR DIPALMITOYL PHOSPHATIDYLCHOLINE VESICLES. J. Suurkuusk*, B.R. Lentz, Y. Barenholz*, R.L. Biltonen*, and T.E. Thompson. Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia, 22903.

The results of a calorimetric and fluorescent probe study of the thermotropic behavior of various types of dispersions of dipalmitoyl phosphatidylcholine bilayer vesicles will be reported. Bangham-type, multilamellar vesicles exhibit two distinct phase transitions at 34.6° and 41.2°. On the other hand, single-lamellar spherical vesicles appear to exhibit a single transition at 37°. The single-lamellar vesicles are thermodynamically unstable below 27° and slowly transform into a multilamellar structure with a single phase transition of 41.2°. These transformed structures resemble, but are not identical to, Bangham-type vesicles. An experimentally testable thermodynamic and kinetic model based upon these results has been developed.

**F-POS-H14** DEPENDENCE OF SIZE DISTRIBUTIONS OF PHOSPHOLIPID VESICLES ON LIPID COMPOSITION. J.H. Goll*, Biophysics Department, Johns Hopkins University, Baltimore, Md. 21218, Y. Barenholz* B.J. Litman, and T.E. Thompson, Biochemistry Department, University of Virginia Medical School, Charlottesville, Va. 22901

Photon correlation spectroscopy (PCS) has been used to investigate the influence of lipid composition on the size of phospholipid vesicles. Prolonged ultracentrifugation was used in order to obtain a relatively uniform population of small, single-walled vesicles. The vesicle size distributions were obtained by fitting the PCS data directly to a parameterized distribution function using the method of splines. Vesicles were prepared from egg phosphatidylcholine (PC), beef brain sphingomyelin (SPM), mixtures of egg phosphatidylethanolamine (PE) and PC, and PC-SPM mixtures. The size variations among all compositions were small, the sizes falling generally within the range of 100-140 Å radius. Egg PC formed vesicles with a narrow distribution centered near 105Å. Pure SPM vesicles were similar. Both mixed composition systems showed systematic increases in size with decreasing PC content and maximum polydispersity at intermediate PC concentration. These trends are believed to reflect the packing properties of the lipids within the bilayer. This research was supported by USPHS grants AM 12803, AM 16315, GM 05181, GM 14628, HL 17576, and NSF grant GB 41313.

**F-POS-H15** NMR RELAXATION RATE STUDY OF THE EFFECT OF THE GENERAL ANESTHETIC HALOTHANE ON A MODEL MEMBRANE SYSTEM CONTAINING CHARGED LIPIDS. Rob G. Parrish, and Robert J. Kurland, Department of Chemistry, State University of New York at Buffalo, Buffalo, New York 14214.

Sodium-23 and Phosphorus-31 pulsed nmr measurements have been carried out on lamellar lipid bilayer systems consisting of aqueous dispersions of the cephalin fraction from beef brain with varying concentrations of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) present. The cephalin fraction was a mixture of phosphatidylethanolamine and the charged lipid, phosphatidylserine, present in roughly equal amounts, and, as minor components, several phosphatidylinositides. The sodium-23 results show that $Na^+$ exchanges rapidly between the bulk solution and sites at the membrane surface (at 28°C); physiologically relevant concentrations of halothane decrease the sodium relaxation rates, a result which is consistent with decreased binding of $Na^+$ to the membrane. The phosphorus-31 results indicate that the anesthetic causes greater local mobility of the phosphate group at temperatures below 10°C, although not necessarily greater fluidity of the membrane as a whole. We thank Dr. Demetrios Papahadjopoulos and Dr. Ross Markello for helpful discussions and for much of the materials used.

**F-POS-H16** ANALYSIS OF FORCES BETWEEN PHOSPHOLIPID BILAYERS. V. A. Parsegian, National Institutes of Health, Bethesda, Maryland 20014, A. C. Cowley*, and R. P. Rand, Brock University, St. Catharine's Ontario.

We have extended measurements of bilayer repulsion using the "osmotic stress" technique (LeNeveu et al. Bioph. Soc. Abs. #W-Pos-B17 (1975)). The repulsion between lecithin bilayers follows an exponential law $P_o$ exp (separation/1.93 Å), $P_o = 10^{11}$ dyne/cm$^2$. At 27.5 $\pm$ .5 Å separation, repulsion is balanced by a van der Waals attraction of 5 to 8.5 x $10^4$ dyne/cm$^2$ (obtained by equating attraction to the repulsion force extrapolated to the range of equilibrium separation). This attraction compares well with computed van der Waals forces provided one recognizes that the bilayer has a distinct polar group-plus-water layer on either side of the lipid hydrocarbon. Modification of this force by adding sucrose to the intervening water layers causes spacing changes of 5 to 6 Angstroms. Changes of this magnitude are also in accord with theoretically predicted shifts in equilibrium separation. We are now measuring long-range forces between charged bilayers.

F-POS-11 COMPARISON OF LIGHT MEROMYOSIN FROM BREAST MUSCLE OF NORMAL AND DYSTROPHIC CHICKEN. E.B. McGowan, S.A. Shafiq*, and A. Stracher, Depts. of Biochemistry & Neurology, SUNY Downstate Med. Ctr., Brooklyn, N.Y. 11203

Amino acid composition, ATPase activity, and ultracentrifugal analysis of myosin from normal and dystrophic chickens and mice did not show significant differences (Barany et al, Ann. NY Acad. Sci. 138, 360 (1966); Morey et al, Arch. Biochem. Biophys. 119, 491 (1976)). Differences in normal and dystrophic mouse myosin were shown by SDS gels (John et al, J. Neurol. Sci. 18, 421 (1973)). We have found that SDS gels of myosin prepared from adult dystrophic chickens show several more protein bands than gels of myosin prepared from normal chickens of the same age. Light meromyosin (LMM) from the two types of muscle was prepared using tryptic cleavage and alcohol precipitation. Paracrystals obtained by dialysis of 0.1-0.5 mg/ml protein solns. were examined by light and electron microscopy. Paracrystals from normal chicken LMM showed periodicities typical of fast muscle. LMM from older dystrophic chickens gave aggregates mostly lacking periodicity; LMM from younger dystrophic chickens gave paracrystals often showing periodicities different from those observed in normal muscle. Whether these changes represent defective formation of myosin in dystrophic muscle or a secondary degeneration of it remains to be determined.

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F-POS-12 THE RESPONSE OF ACTIN TERTIARY STRUCTURE TO S-1 BINDING. J. Loscalzo*, G.H. Reed*, and A. Weber. Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19174.

Changes in the conformational state of an actin monomer in response to myosin (S-1) binding were monitored using the spin-spin interaction between covalently bound spin label, 3-(maleimidomethyl)-2,2,5,5 tetramethyl-1-pyrrolidinoxyl, and $Mn^{2+}$ bound unexchangeably to each monomer in an actin filament. The response was biphasic, first decreasing then increasing with increasing S-1 binding. This change in spin-spin interaction is attributed to a change in distance between both labels, with or without an accompanying change in orientation, for the following reasons. (1) The correlation time for the interaction between the spin label and $Mn^{2+}$ did not change significantly with increasing S-1 binding. (2) Analysis of simulated spin label ESR spectra demonstrated that a change in relative orientation of spin label and $Mn^{2+}$ could alter the magnitude of the interaction, but not to the extent observed experimentally. Experiments with copolymers of labelled and unlabelled monomers showed that the change in spin-spin interaction occurred within, not between, monomers. It is concluded that this phenomenon most likely reflects a change in monomer conformation propagated along the filament from complexed to uncomplexed monomers in response to S-1 binding. It should be emphasized that the actin used was free of tropomyosin. This work was supported by NIH grants GM02046, AM17517 and HL15835 and NIAMDD grant AM70134.

F-POS-13 PARAMYOSIN IDENTIFICATION AND PARAMYOSIN/MYOSIN HEAVY CHAIN MOLECULAR RATIOS IN INVERTEBRATE MUSCLES. R.J.C. Levine, M. Elfvin, and M.M. Dewey, Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, Pa. 19129 and Department of Anatomical Sciences, SUNY, Stony Brook, N.Y. 11794.

We have previously shown that a band, coelectrophoresing with purified Limulus paramyosin (PM) chains is found in sodium-dodecyl sulfate (SDS) polyacrylamide gels of a variety of glycerinated invertebrate muscle preparations. Here we report (1) the identification of this band as PM chains by immunodiffusion and (2) the determination of the PM/myosin heavy chain (MHC) ratios in these muscles. Antibodies against purified Limulus PM which had been denatured by boiling in 0.01M sodium phosphate buffer, pH 7.0, containing 0.1% SDS and 0.1% dithioerythritol formed precipitin lines vs. PMs eluted from gels (Lazerides, J. Cell Biol. 65: 549, '75) of Mercenaria opaque adductor and Homarus claw muscle homogenates, confirming the crossreactivity of PMs indicated by our earlier immunofluorescent studies. Using quantitative SDS-polyacrylamide gel electrophoresis we have determined that Coomassie Brilliant Blue binding by both PM and MHC is a linear function of loading, up to 10 µg of PM and 14.5 µg of MHC, respectively. A factor for converting dye-binding ratios to molecular ratios was derived from these curves. PM/MHC dye binding ratios obtained from the integration records of gels loaded with between 2.5 µg and 20 µg of total muscle protein per muscle type were converted into molecular ratios. The results of these studies support a positive correlation between PM content and filament dimensions in invertebrate muscles.

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**F-POS-I4** PROPERTIES OF THE ISOLATED COMPONENTS OF BOVINE CARDIAC TROPONIN. L.D. Burtnick*, W.D. McCubbin*, and C.M. Kay, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Cardiac troponin can be separated into three protein components by a combination of ion exchange chromatographies on DEAE- and CM-Sephadex in the presence of 6 or 8 M urea. These components include: TN-C, calcium binding protein; TN-I, an ATPase inhibitory protein; and TN-T, which interacts with tropomyosin. Sedimentation equilibrium data suggest molecular weights for cardiac TN-C, TN-I and TN-T of 17700 ± 1000, 22900 ± 500 and 36300 ± 200, respectively. Circular dichroism studies show that cardiac TN-C undergoes a major conformational change upon binding $Ca^{2+}$. The three troponin components, when added to a synthetic cardiac actomyosin assay system, in the presence of tropomyosin, reconstitute a $Ca^{2+}$-sensitive relaxing system. Circular dichroism, sedimentation velocity, and polyacrylamide gel experiments reveal that interactions may occur between TN-C and TN-I, TN-C and TN-T, and TN-T and tropomyosin. These results support the hypothesis that regulation of cardiac muscle contraction occurs, qualitatively, in the same manner as that proposed for striated muscle.

**F-POS-I5** STUDIES ON 'M-PROTEIN' FROM CHICKEN MUSCLE. J. Trinick* and S. Lowey. (Intr. by C. Cohen). Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154.

M-protein (170,000 daltons) is extracted from chicken muscle by Hasselbach-Schneider solution. Such extracts also contain a second 170,000 component which purifies with the M-protein through fractionations in ammonium sulphate (32% saturated) and low salt (10mM KPi). On SDS gels both proteins migrate together between myosin and C-protein in the band designated 'b' by Starr and Offer in their 1971 classification of myosin contaminants. Separation of the two proteins is achieved by ion exchange chromatography on DEAE cellulose (50mM Tris-HCl, pH 8). The proteins are distinguished by their sedimentation coefficients. The M-protein sediments at 5.1S, whereas the value for the other component is 7.1S. They are similar in that they are both low in α-helix. The magnitudes of the troughs at 208nm in the circular dichroism spectra indicate that the M-protein has no α-helix while the 7.1S component is about 17% helical. Correspondingly, their amino acid compositions are similar and both are relatively high in proline. Rabbit antibodies to each of the proteins were characterised by double diffusion in agar. Precipitin lines formed only between the proteins and their homologous antibodies. Moreover, the line resulting from one reaction did not fuse with that from the other. Thus, the two proteins do not appear to have common antigenic determinants. Binding of fluorescein coupled antibodies to myofibrils was studied by phase contrast and fluorescence microscopy. As expected, the M-protein antibodies strongly stained the middle of the A-band. In contrast, antibodies to the 7.1S component weakly stained the Z-line region.

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**F-POS-I6** EFFECT OF $Mg^{++}$ ON MYOSIN B's CaATPase ACTIVITY. G.W. de Villafranca, E.H. Foran, and C.C. Myers, Dept. of the Biol. Sciences, Smith College, Northampton, Mass. 01060.

We have reported that the effect of $Mg^{++}$ on Limulus myosin B ATPase is varied and that consequently it is difficult to investigate $Ca^{++}$ regulation of the MgATPase. These variations from one preparation to another led us to a systematic approach to myosin B preparation in an attempt to obtain a reproducible system.

Purified myosin B, without paramyosin or tropomyosin, prepared by direct extraction of Limulus muscle consistently yields an ATPase with high activity in the presence of about 4 mM $CaCl_2$ which is lowered by the addition of $MgCl_2$ to about 1 mM; that is, a $Mg^{++}$ sensitivity. Activity is even lower with 1 mM $Mg^{++}$ alone while the addition of $Ca^{++}$ does not increase the activity substantially: that is, the activity with $Mg^{++}$ plus $Ca^{++}$ is not as high as with $Ca^{++}$ plus $Mg^{++}$.

On the other hand, when muscle myofibrils are washed with 0.05 M KCl, 5 mM phos. buffer, 1 mM $MgCl_2$ and 0.1 mM DTT alone, or with 0.1 mM EGTA added, or with 0.1 mM EDTA added (the latter a better relaxing medium for Limulus), the myosin B extracted with 0.6 M KCl, 1 mM ATP, 0.1 mM DTT and 5 mM phos. buffer, pH 7.5 has a CaATPase which is slightly enhanced by Mg addition; that is, it is not $Mg^{++}$ sensitive. Again, activity with $Mg^{++}$ alone or with $Mg^{++}$ plus $Ca^{++}$ is still below that when $Ca^{++}$ plus $Mg^{++}$ are present. These preparations do exhibit $Ca^{++}$ sensitivity as defined by the EGTA measure. When the myosin B is washed with histidine or tris buffers, as much as 30% of the protein is removed and the washed myosin B loses some Ca sensitivity and becomes $Mg^{++}$ sensitive. SDS gels demonstrate that specific proteins are removed by washing and that a new protein appears.

**F-POS-I7** LIGHT DIFFRACTION PATTERN ANALYSIS OF SINGLE SKELETAL MUSCLE FIBERS AND FIBER BUNDLES. P.J.Paolini, Dept. of Biology, San Diego State University, San Diego, CA 92182, R.A.Sabbadini*, Laboratory of Physiology and Biophysics, University of the Pacific, San Francisco, CA 94115, K.P.Roos* and R.J.Baskin, Dept. of Zoology, University of California, Davis, CA 95616.

Light diffraction patterns produced by single skeletal muscle fibers and small fiber bundles of Rana pipiens semitendinosus have been examined at rest and during tetanic contraction. The muscle diffraction patterns were recorded with two types of position-sensitive light diffractometers. The first instrument utilized a vidicon CCTV camera interfaced to a minicomputer through a programmable controller. The second diffractometer employed two lag-free 256 element linear image sensors to monitor two or more diffraction lines. First order line intensity and peak amplitude exhibited maxima at about 3.0 u; this behavior can be explained in terms of simple scattering theory. Digitized video output from either instrument was analysed on-line to evaluate mean sarcomere length (SL) from the line spacing, the line intensity, and the sarcomere length dispersion (%D) from first order line widths. Measurements made along the length of single fibers exhibit small variations in calculated mean SL (s.d. 1.2%) and %D (2.1% ± 0.8%). Small fiber bundles show a linear dependence of %D upon fiber number (approx. 0.2%/fiber) to a maximum of about 10% in large bundles or whole muscles. The first order line intensity decreases by about 40% during tetanus; larger multifibered bundles show significant increases in dispersion during contraction, while single fibers do not.

**F-POS-I8** A MODIFICATION OF THE DMO TECHNIQUE FOR DETERMINATION OF INTRACELLULAR pH IN FROG SARTORIUS. B. Graves and R.D. Moore. Biophysics Laboratory, State University of New York, Plattsburgh, 02901.

Intracellular pH, $pH_i$, has been determined by a modification of the DMO (5,5-dimethyl-2,4-oxazolidinedione) technique which gives a more accurate estimate of intracellular $^{14}$C-DMO. This technique is based upon the finding that more that 99.4% of tissue (Sartorius of Rana pipiens) $^{14}$C-DMO readily diffuses out of 40 - 60 mg muscles in 2 hr and more than 99% of the remainder is washed out during an additional 10 hr. The wash-out kinetics are fit by a 3-compartment model. The time constant, $t_{1/2}$, for the 1st component ranges from 0.8 to 1.6 min., for the 2nd component from 5.1 to 10.8 min., and for the slowest component from 34 to 90 min. The first component is considered to represent extracellular space, ECS, and the 2nd to represent cytoplasm. The third component likely represents a bound fraction and in any case represents less than 10% of the tissue DMO. The components were separated by a least-squares curve-peel technique.

The $pH_i$ of 12 frog sartorii were determined by this method and simultaneously determined by using $^3$H-sucrose as a marker for the estimation of ECS. $pH_i$ determined by the modified $^{14}$C-DMO method averaged 7.17 ±0.07 (S.E.) as compared to 7.00 ± 0.08 (S.E.) when estimating intracellular $^{14}$C-DMO using $^3$H-sucrose. The value 7.17 is very close to the value 7.12 obtained with intracellular ipH-selective microelectrodes by Kostyuk et al. (1969, in "Glass Microelectrodes" ed. by M. Lavelle, O.F. Schanne and N.C. Hebert, pp 322-348, Wiley, N.Y.). Use of 14.5 mM tris as buffer resulted in an increase of $pH_i$ of 0.45 units compared to phosphate buffer. In phosphate and bicarbonate buffer, addition of 5% $CO_2$ lowered $pH_i$ by about 0.3 units.

**F-POS-I9** COMPUTER SIMULATION OF TIME DEPENDENT CONTRACTILE PATTERNS IN THE GUINEA PIG ATRIUM. R.B. Robinson+ and W.W. Sleator, Dept. of Physiology and Biophysics, Univ. of Ill., Urbana, Ill. 61801

The Plato IV computer-based education system at the University of Illinois, Urbana, was used to develop and explore a model of calcium (Ca) movements in the guinea pig atrium as a function of stimulation history. The computer program can solve up to eight simultaneous differential equations by numerical integration using a modified Euler predictor-corrector method. A stimulus is simulated by transiently increasing selective rate constants. The peak Ca level shortly after the stimulus in the site representing the myofibrillar space is taken as being proportional to peak contractile tension. In this manner various tension-interval, or restitution, curves can be generated. Previous work on the restitution process suggested that there are at least two sites of contractile Ca in the guinea pig atrium, one of which is blocked by the neutral alkaloid ryanodine (Rd). This computer analysis has demonstrated that a simple model based on Rd sensitive and Rd insensitive sites is not sufficient to fully explain the interval dependence of contraction observed experimentally. It appears that additional sites are needed to serve as buffers, and it is also necessary to incorporate a dependence on recent stimulation history into one or more of the rate constants. Such rate constants would thus have different values when the model simulated different steady-state contraction frequencies. The model should provide insight into how various agents (e.g. D600, TTX) might induce specific alterations in the restitution curve. The possibility of certain model parameters being dependent on action potential configuration is also being investigated. (Supported by Grant No. 13349 from Nat. Heart-Lung Inst.)

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**F-POS-110** THE EFFECT OF $Mg^{2+}$ ON THE BINDING OF $Ca^{2+}$ TO GLYCEROL-EXTRACTED MUSCLE FIBERS. Franklin Fuchs and Margaret Bayuk*, Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15261.

Binding of $^{45}Ca^{2+}$ to glycerol-extracted rabbit psoas fibers (sarcomere length 2.5$\mu$m) was measured by means of a double isotope technique in which $^{3}H$-glucose was used as a marker for solvent space. [$Ca^{2+}$] was controlled by EGTA buffers. With 4mM $Mg^{2+}$ (no ATP) $Ca^{2+}$ binding was half-maximal at pCa 5.89 and the amount bound at saturation was 1.61 $\pm$ 0.08$\mu$moles/gm protein. At $<$50% saturation the Scatchard plot had a positive slope and the Hill coefficient was 2.2. At$>$ 50% saturation the slope of the Scatchard plot was negative and linear ($K' = 10^{5.9}$ $M^{-1}$) and the Hill coefficient was 1.04. In the absence of $Mg^{2+}$ half saturation occurred at pCa 6.52 and the maximum bound $Ca^{2+}$ was 2.89 $\pm$ 0.13$\mu$moles/gm protein. The Scatchard plot indicated two classes of sites with K' values of $\sim 10^{7.5}$ $M^{-1}$ and $\sim 10^{6}$ $M^{-1}$. The Hill plot was linear over the middle range of the saturation curve with a coefficient of $\sim$0.7. The data indicate that $Mg^{2+}$ 1) suppresses binding to about half of the total $Ca^{2+}$ binding sites, and 2) imposes a strong positive cooperativity on half of the remaining sites. (Supported by NIH grant AM 10051 and a grant from the Muscular Dystrophy Association).

**F-POS-111** SKELETAL MUSCLE F-ACTIN AS AN INHIBITOR OF EMBRYONIC MICE FIBROBLAST AGGREGATION. J. Borejdo*, A. Muhlrad*, H. Gadasi*, and A. Oplatka, Department of Polymer Research, The Weizmann Institute of Science, Rehovot and Department of Cellular Biochemistry, Hebrew University Medical School, Jerusalem, Israel.

Actin and myosin have recently been found in association with non-muscle cell plasma membrane and it has been suggested, especially with reference to fibroblasts, that interaction between these proteins plays a role in cell adhesion and aggregation. We therefore investigated the effect of rabbit skeletal F-actin on the aggregation of embryonic mice fibroblasts (BALB/c, C57BL/6 and C3H). The cells were suspended by either trypsin or EDTA treatment in Way's medium (0.5-1.0x$10^{6}$ cells/ml) and F-actin (0.01-200 µg/ml) was added. The suspension was incubated in a gyrotary shaker and the rate of decrease in optical density at 660 nm was taken as a measure for the rate of aggregation. After one hour, the rate of aggregation was diminished by a factor of about 15 in the presence of 100 µg/ml actin and 200 µg/ml were enough for the complete inhibition of aggregation. 0.01-10 µg/ml of actin did not exert any observable effect. Bovine serum albumin, natural tropomyosin and rabbit skeletal heavy meromyosin at concentrations of up to 1 mg/ml had no effect on aggregation. Protein determination in the supernatant after sedimentation of the cells showed that only actin was adsorbed to the suspended fibroblasts. It appears that surface alterations induced by bound actin could be utilized for controlling cellular adhesivity.

**F-POS-112** SINGLE BEAT ESTIMATION OF LEFT VENTRICULAR SOURCE CHARACTERISTICS DURING SYSTOLIC EJECTION. J. Berry Jr., D.E. Downie, and B.R. Wilcox‡ Biomedical Engineering & Mathematics, Division of Cardiothoracic Surgery, University of North Carolina, Chapel Hill, N.C. 27514

The dynamic behavior of the left ventricle can be described in terms of a fluid Thevenin equivalent, i.e. a hydrodynamic driving pressure $P_S$ in series with a source resistance $R_S$. The load $R_L$ for this left ventricular representation is the systemic resistance. For $R_S/R_L$ large ($>1$), the ventricle behaves more like a flow source. For $R_S/R_L$ small ($<1$), it behaves more like a pressure source. Previous studies have characterized the left ventricle as a flow source (Buonchristiani et.al., IEEE Trans. Biomed. Eng. 20 110 (1973)), and as a Thevenin equivalent whose source impedance is matched to the systemic load (Elzinga and Westerhof, CIBA Symposium 24 (new series) 241 (1974)).

A new approach to this controversial question is presented. A computer simulation model of the heart (Downie et.al., Proc. 28$^{th}$ ACEMB, 433 (1975)) is used to estimate, during systolic ejection, the time dependent Thevenin equivalent of the left ventricle. An advantage of this method is that data from only a single cardiac cycle is needed. Preliminary results from animal studies indicate that the left ventricle is more pressure-like during ejection, especially in the early stages. Furthermore, the source character appears strongly dependent on aortic valve timing, simulation of opening delay lowering $R_S$ (more pressure-like). Simulated contractility changes also effect the Thevenin equivalent. Increases in Vmax had little effect on $P_S$, lowered $R_S$, and thus increased the pressure-like character. Increases in Tmax increased $P_S$ but had little effect on $R_S$, thus changing the strength but not the character of the source. Finally, simulated increases in EDV decreased $R_S$ and increased $P_S$, thus increasing both strength and pressure-like character of the left ventricle.

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F-POS-113 EFFECT OF ELECTRON-ATTRACTIVITY OF HEME SIDE CHAINS OF MYOGLOBIN ON EQUILIBRIUM AND KINETIC CONSTANTS FOR OXYGEN AND CARBON MONOXIDE. M. Sono,[a]* P.D. Smith,[c]* J.A. McCray,[b] and T. Asakura,[a] [a]The Departments of Pediatrics and Biochemistry, Children's Hospital of Philadelphia and Univ. of Pa., [b]the Department of Physics and Atmospheric Science, Drexel Univ., and [c]the Johnson Research Foundation, Univ. of Pa., Philadelphia, Pa. 19104.

Effects of chemical modifications of heme on $O_2$ and CO binding of horse heart myoglobins (Mb) containing six different hemes were investigated. The combination("on") rate constants for $O_2$(k') and CO(1') obtained by laser flash photolysis experiments in 0.1 M phosphate buffer, pH 7.0 at 20° were, k'=0.83(meso-), 2.4(deutero-), 1.1(reconst. proto-), 1.2(native proto-), 1.5(2-formyl-4-vinyl-), 1.9(2-vinyl-4-formyl-), and $2.7\times10^7$ $M^{-1}s^{-1}$(2,4-diformylmyoglobins) and 1'=2.8, 18, 4.8, 5.1, 7.1, 15 and $35\times10^5$ $M^{-1}s^{-1}$ in the same order. These rate constants, except for those of deuteromyoglobin, increase in the order of increasing electron-withdrawing power of the side chains, indicating that reduced electron density of the iron atom of heme favors the "on" reactions with $O_2$ and CO. Equilibrium constants(L) for association of various Mbs with CO, determined by the partition coefficients(M) between $O_2$ and CO, also increased with an increase in the electron-attractivity of the heme side chains. Although this order is completely opposite to the case of the $O_2$ binding reaction(Sono & Asakura, J. Biol. Chem. 250, 5227 (1975)), the dissociation("off") rate constants for $O_2$(k) and CO(1), calculated from the equilibrium and the "on" rate constants, showed a similar behavior as the "on" rate constants. The concomitant increase in both "on" and "off" rate constants with the increase in electronegativity of the iron atom, suggests that the "on" and "off" reactions have different rate determining steps. In the "on" reaction σ-bond formation appears to be dominant while in the "off" reaction π-bond break-up is more important. Supported by NIH HL-18226, HL-16734 and NHLI-72-2962B. P.D.S. by NIH:AM-02762B and J.A.M. by NSF BM575-01623.

F-POS-114 EQUILIBRIUM CENTRIFUGATION OF OVALBUMIN IN AQUEOUS ACETIC, PROPIONIC AND BUTYRIC ACIDS. S. Szuchet, Department of Biophysical Sciences, State University of New York at Buffalo, Buffalo, NY 14226.

The tendency of ovalbumin to irreversibly aggregate when dissolved in acid solutions of moderate to high ionic strength has been reported. Here I report the behaviour of ovalbumin in aqueous solutions of short-chain aliphatic acids in the absence of any other added electrolyte; i.e., at very low ionic strength. These solutions are highly nonideal because of the extreme Donnan effect. Under these experimental conditions ovalbumin exhibits also a tendency to self-association. However, the extent and reversibility of these associations appear to be related: first, to the length of the hydrocarbon chain; second, to the concentration of acid used. Of the three acids studied - acetic, propionic and butyric - acetic acid appeared to be the better solvent for ovalbumin. For example, in 0.1 M acetic acid ovalbumin behaved as a single thermodynamic component with indication of a reversible monomer-dimer association taking place in this solvent. In 1 M and 10 M acetic acid, on the other hand, a small amount of heterogeneity was evident. Again, different extent of reversible association had to be postulated in order to account for the experimental data in these solvents. The extent of irreversible aggregation increased in propionic and butyric acids. In the latter solvent considerable precipitation occurred in 1 M solution and several attempts to dissolve ovalbumin in 10 M butyric acid proved unsuccessful.

F-POS-115 PHOSPHOROUS NMR STUDIES OF ALKALINE PHOSPHATASE. D.P. Hollis, H. LeVine III,* and R.L. Nunnally,* Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205

The phosphorous-31 NMR spectra of 2 mM native E. Coli (C-90 strain) alkaline phosphatase, 4mM $HPO_4^=$ have been obtained in the pH range of 5.2-7. and at temperatures of 15°, 25°, and 31° C. Approximate longitudinal relaxation times have been measured at pH 5.2, T=31°C using the inversion recovery method. Three distinct phosphate resonances were observed in each case having chemical shifts of approximately 8.9, 3.7, and 0.7 ppm from external phosphoric acid depending somewhat on the pH and temperature. The corresponding relaxation times are $T_2=1\times10^{-2}$, $5\times10^{-3}$, and $2\times10^{-3}$ sec. and $T_1$= .75, 0.3 and 0.2 sec. The Cd analog of alkaline phosphatase was also examined at pH 6.4, 31°C. It showed only two phosphate resonances at about 1.3 and 13.1 ppm from the reference. We have tentatively assigned the two lower field resonances to covalently bound phosphate because of their large shifts and relatively small linewidths. The peak at highest field is assigned to non-covalently bound phosphate in rapid exchange with free phosphate. That the $T_2$'s are much less than the $T_1$'s suggests that exchange among environments of different chemical shifts may be responsible for the observed linewidths. Spin-spin coupling was shown to make only a minor contribution to the linewidths. At low temperature and pH 5.9 the broad upfield peak dominates the spectrum. As the temperature is raised this resonance sharpens dramatically. At high pH however it becomes very broad. In addition, low pH favors the low field covalent phosphate resonance while high pH favors the high field covalent phosphate resonance. These temperature and pH effects are reversible. Possible relationships between these results and the postulated covalent enzymatic intermediates will be considered. (Supported by NIH Grant GM 17172).

F-POS-116 STUDIES OF ANS-LABELLED CHYMOTRYPSINOGEN AND CHYMOTRYPSIN. J. David Johnson * and M. Ashraf El-Bayoumi, Dept.of Biophysics, Mich. State Univ. E. Lansing, MI. 48824

The fluorescent probe molecule 8-Anilino-1-Naphthalene Sulfonate (ANS) has an intense blue fluorescence in non-polar solvents or when bound to non-polar crevices of proteins, yet it is practically non-fluorescent in aqueous solutions. (L. Stryer, J. Mol. Biol.13,482, 65, D.C. Turner and L. Brand, Biochem.7, 3381,68.) This probe molecule binds to Chymotrypsinogen (CG) and Chymotrypsin (CHT) at low pH and fluoresces intensely with an emission maxima near 480 nm. As the pH is raised from 3.5 to 8.0 the relative fluorescence is observed to decrease dramatically for both CHT and CG. The binding of ANS to CHT does not affect CHT activity and competitive inhibitors such as β -phenylproprionate and tosylation of CHT do not affect ANS binding to CHT. These results suggest that ANS is bound to a pH-dependent hydrophobic site other than the active site of CHT and that this site exist in a modified form in the zymogen,CG. At pH 7.0 CG-ANS fluorescence is five times more intense than CHT-ANS fluorescence. Upon tryptic conversion of CG to CHT the CG-ANS fluorescence reduces to the fluorescence of the CHT-ANS complex. This allowed us to moinitor the activation of CG to CHT. The kinetics of the activation as determined by the decrease in fluorescence at 490 nm are identical to the kinetics of the tryptic conversion of CG to CHT as determined by other means. We have recently allowed ANS to diffuse into CHT crystals at pH 3.6, producing highly fluorescent CHT crystals. X-ray analysis of these labelled crystals is being completed in Prof. A. Tulinsky's laboratory , Michigan State University.
Supported by funds from NIH Training Grant #GM01422 and the College of Osteopathic Medicine of Michigan State University.

F-POS-117 A STUDY OF THE N-BROMOSUCCINIMIDE INACTIVATION OF THE ENZYME RHODANESE. K. Guido*, and P.M. Horowitz, Department of Chemistry, Dartmouth College, Hanover, N.H. 03755

The enzyme rhodanese (EC 2.8.1.1) is rapidly inactivated by treatment with N-bromosuccinimide (NBS) in either 0.1 M glycine, pH 6.0 or in 0.1 M Tris, pH 8.0. Spectrophotometric titration at pH 6.0 indicates that tryptophan oxidation is not responsible for the observed effect. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis shows that the inactivation is not due to polypeptide chain cleavage. Sulfhydryl (SH) group assays were performed at pH 8.0 (glycine solutions were adjusted) using the colorimetric reagent 5,5'-dithiobis(2-nitrobenzoic acid) after excess NBS was destroyed by the addition of tryptophan. SDS was added to denature the protein. After initial incubation at pH 6.0 or pH 8.0 the titer of SH groups assayed at pH 8.0 indicated that some SH groups were unavailable after NBS oxidation. As observed previously, the SH group availability in control samples is reduced in the presence of glycine. In Tris, pH 8.0, however, the free sulfhydryl titer is 2.2 SH groups/molecule after NBS oxidation compared with 4.1 SH groups/molecule in a control. Further, NBS inactivated rhodanese can be significantly reactivated (>33%) by incubation with the substrate thiosulfate. It is concluded that NBS can inactivate rhodanese by inducing the formation of a disulfide bond between the known active site sulfhydryl group of the enzyme and a second sulfhydryl group close to the active site in the native structure.

F-POS-118 RELAXATION KINETICS STUDY OF THE INTERACTIONS OF 2', 4', 5', 7' - TETRAIODOFLUORESCEIN (TIF) WITH ASPARTATE TRANSCARBAMYLASE (ATCase). P. C. Keck and T. M. Schuster, Department of Biological Sciences, Univ. of Connecticut, Storrs, Conn. 06268, and C. H. McMurray, Veterinary Res. Labs, Stormont, Belfast, N. Ireland.

We have measured the kinetic relaxation spectrum of the interactions between TIF and ATCase by following changes in absorbance at 546 nm subsequent to a temperature jump from 19 to 23°C at pH 8.3 in .1 M Hepes buffer. The relaxation spectrum consists of three phases having relaxation times in the range of 0.2, 7, and 30 msec. The rate of the fastest phase increases with increasing protein concentration at low, constant, TIF saturation. Under these conditions, addition of 10 mM ATP or 3 mM CTP lowers both the rate and the amplitude of the fastest phase; The rate of the slowest phase, however, is independent of protein concentration at low, constant, TIF saturation, but increases at higher TIF saturation. These effects are interesting in light of the results of initial rate and equilibrium binding studies (Jacobsberg, L.B., Kantrowitz, E.R., and Lipscomb, W.N., to be published in JBC in late 1975) which show, respectively, that at low saturation TIF activates the enzyme and that ATP and CTP each compete for six TIF binding sites.
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