F-POS-E1 HUMAN LYMPHOCYTES: ELECTROPHORETIC MOBILITY MEASUREMENTS AND DETECTION OF SURFACE REACTIONS BY LASER DOPPLER SPECTROSCOPY. J.H. Kaplan* and E.E. Uzgiris, General Electric Research and Development Center, Schenectady, New York 12301.

The changes in electrophoretic mobility and isoelectric point produced by incubating human peripheral blood lymphocytes with phytohemagglutinin (PHA) and concanavalin A (Con A) have been characterized by laser Doppler spectroscopy, a technique which allows for the measurement of electrophoretic mobilities through the measurement of the Doppler shifts of laser light scattered from suspended particles that have been subjected to an electric field. The results extend and partially confirm older observations made by classical procedures. Incubation with either agent for 90 minutes at 37°C resulted in stable and reproducible decreases in electrophoretic mobility, and increases in the isoelectric point. The incubation conditions used are known to permit primary attachment of the phytomitogen, capping and endocytosis; nevertheless, at least in the case of Con A, washing the cells with a specific inhibitor for Con A binding, methyl-α-D-glucoside (MAG), resulted in complete reversal of the electrokinetic changes, showing that the underlying changes in cell surface constitution detected under these conditions are solely due to reversibly bound Con A. The results suggest that laser Doppler spectroscopic changes could provide a direct assay for specific binding to immunocompetent cell surfaces.

Supported in part by the Molecular Control Program of the National Cancer Institute under Contract N01-CP-3-3231.

F-POS-E2 OBSERVATION OF PROTOPLASMIC STREAMING BY LASER-LIGHT-SCATTERING. R. V. Mustacich* and B. R. Ware, Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138.

Data are presented which demonstrate that the laser light scattered from the protoplasm of living cells of Nitella flexilis is Doppler shifted by the streaming motion of the macromolecules and particles in the protoplasm. The laser-light-scattering spectra are used to determine the velocities and velocity distributions inside the cells. Collection of these spectra is very precise, perfectly objective and extremely rapid. Both the Doppler shift and the linewidth are shown to be a linear function of the scattering vector. The observed temperature dependence of the velocity shows positive curvature. At temperatures above 34°C, irreversible cessation of the streaming results. The temperature dependence of the halfwidths of the velocity distributions is approximately linear and extrapolates to a positive intercept at 0°C. The dependences of the Doppler spectrum on cell orientation, scattering angle, and cell temperature are used to infer details of the streaming pattern. Brownian motion of the scattering particles is shown to be negligible.

F-POS-E3 TRANS ELECTROPHORESIS OF BIOMOLECULES AND CELLS
Nicholas Catsimpoolas, Biophysics Laboratory, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

A new instrumentation system has been developed for the kinetic study of the transport and diffusion processes of charged biomolecules and bioparticles in an electric field. The transport path is monitored by repetitive electro-optical scanning in the uv and visible region and the absorbance or scattering of light is digitized and processed by a computer. This technique allows the use of statistical moment and slope analysis (first and second derivative) for quantitative evaluation of the transient state (TRANS) of the charged species distribution. The electrophoretic methods employed in this study include continuous-pH zone electrophoresis (CZE), multiphasic zone electrophoresis (MZE), isotachophoresis (ITP), and isoelectric focusing (IF) in density gradients and polyacrylamide gels. Cells have been analyzed primarily by CZE in Ficoll gradients and proteins by all methods. Parameters that have been measured include electrophoretic velocity, relative mobility from velocity measurements, retardation coefficient in gels, apparent diffusion coefficient, isoelectric point, resolution, the electrofocusing parameter pE, segmental pH gradient, minimal focusing time in IF, and band spreading in electrophoresis as a function of time. (Supported in part by National Cancer Institute, Contract No. NO1-CB-43928).

F-POS-E4 CLASSIFICATION OF HUMAN LEUKOCYTES BY MULTIANGLE LASER LIGHT SCATTERING IN A FLOW SYSTEM. G. C. Salzman, J. M. Crowell,* J. C. Martin,* P. M. LaBauve,* and P. F. Mullaney,* Biophysics and Instrumentation Group, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87544.

Unfixed, unstained human leukocytes have been physically separated into three morphologically distinct populations based only on the intensity of laser light (488 nm) scattered by individual cells in a flow-system cell sorter at two angles with respect to the laser beam (1.0° and 90° with acceptance angles of 0.1° and 25°, respectively) [to be published in the Proceedings of Electro-Optics '74 West and International Laser Exposition (November 5-7, 1974), Industrial and Scientific Conference Management, Inc., 222 West Adams Street, Chicago, Ill. 60606]. Blood samples were prepared by a modified buffy coat procedure, and the erythrocytes were lysed with saponin. The three populations were identified after sorting by fixing the sorted fractions in methanol, staining them with Wright-Giemsa stain, and examining them microscopically. The sorted groups consisted of neutrophils, monocytes, and lymphocytes. Each group was more than 77% pure. Blood from irradiated monkeys was also separated by this technique and contained an eosinophil group (61% purity) as well as neutrophil and lymphocyte groups. Similar results were obtained with a 5 mW helium-neon laser and two angles selected from a photodiode array. A computer-based system in which 32 angles of scattered light intensity can be measured simultaneously for each cell as it passes through the flow chamber has been constructed and will be discussed. (This work was performed under a joint AEC/NCI agreement.)

F-POS-E5 MODIFICATION OF INTENSITY AND DIRECTION OF ELECTRON FLOW ACROSS BILEAFLET MEMBRANES. C-H. Chen* and D. S. Berns, Physical Chemistry Laboratories, Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201.

Black lipid membranes were formed using chloroplast extracts containing chlorophyll, lipid and carotenoids. When a membrane interposed between solutions of different redox potential was irradiated with light, a photo EMF up to 50 mV was recorded. Addition of a biliprotein to the solution on the reducing side of the membrane enhanced the photoresponse by as much as a factor of 2 and shifted the photoelectric action spectrum from that of chlorophyll to reflect a disproportionate contribution of the biliprotein. Eight different biliproteins (phycocyanins, phycoerythrins and allophycocyanins) were successfully tested in these experiments. Using a nominal redox gradient (~2 mV), it was possible to demonstrate that these biliproteins actually direct electron flow. The side of the membrane containing the biliprotein in solution became the reducing side even when it was the oxidizing side prior to the addition of the biliprotein. It has therefore been possible to use an extrinsic membrane protein to modify the photoelectric behavior of chloroplast extract membranes to mimic the energy storage qualities of photosynthesis and solar energy cells. A specific mechanism involving interfacial electron tunneling and transmembrane electron movement has been formulated. The biliproteins interact with the membrane to lower the barrier to allow conventional interfacial movement of the electron over the energy barrier. This work was supported in part by National Science Foundation Grant GB-24315.

F-POS-E6 HISTONE CAN BE A PROBE FOR COUPLING OF CATION TRANSPORT TO ELECTRON TRANSPORT IN MITOCHONDRIA. M. Hillar, D. S. Wong* and D. Stoltz,* Department of Biology, Texas Southern University, Houston, Texas 77004.

Histone (lysine-rich) binds to mitochondria in a saturation kinetics. It does not affect Ca^{2+} uptake or level; it extrudes Mg^{2+} by 30% with the utilization of substrates, with rotenone-by 18%. DNP and ADP prevent the effect of histone. Mg^{2+} uptake is inhibited by 12%. K+ extrusion by histone (by 50%) depends on the oxidation of substrates, is enhanced by phosphate, does not depend on concentration gradient. Mg^{2+} decreases by 50% the K+ extrusion. Histone prevents extrusion of K+ by Ca^{2+}. K+ extrusion is prevented by ADP and low DNP; also histone prevents DNP-induced extrusion of K+. DNP-induced extrusion of K+ is prevented also by substrates. Pi extrudes K+ independently of DNP or substrate. Histone does not affect Na^{+} level, it blocks by 50% Na^{+} uptake. Histone blocks proton uptake by mit., in the presence of valinomycin or DNP. Electron microscope studies reveal that condensed, energized configuration of cristal membrane is converted to orthodox, nonenergized configuration after the addition of histone.

Postulated mechanism of histone action involves immobilization of proton translocation, induction of local changes in H^{+} concentration, prevention of interaction between H^{+} and K^{+} and Mg^{2+} carriers or valinomycin, formation of a specific channeling for Mg^{2+}.

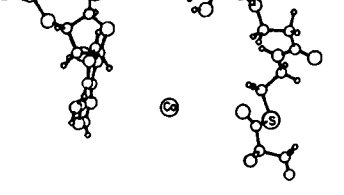
(Supported by NIH 1S06-RR08061.)

F-POS-E7 HIGH FIELD CARBON-13 NMR OF PEPTIDES. R. A. Komoroski, I. R. Peat*, and G. C. Levy*, Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306.

The use of superconducting solenoids in carbon-13 NMR results in a substantial increase in spectral resolution over that obtained at commonly employed magnetic fields. Often ^{13}C peaks which are not resolved at low fields will be resolved when high magnetic fields are employed. Using the partially-relaxed Fourier transform technique, individual spin-lattice relaxation times (T_1) can be obtained for each resolved resonance in the ^{13}C spectrum. Results are presented here for the cyclic decapeptide gramicidin-S in both methanol-d_4 and DMSO-d_6 solutions. In the aliphatic region of the 67.9 MHz ^{13}C spectrum of gramicidin S in CD_3OD, eight additional well resolved resonances are observed. These resonances appear as four unresolved or partially resolved peaks at 15.2 MHz. Thus at 67.9 MHz it was possible to monitor the complete motional behavior of the proline and ornithine side chains using ^{13}C T_1 values, something that could not be done at low field due to overlap of the β and γ carbon resonances of these residues. Information concerning the internal spinning of the magnetically non-equivalent methyl groups of the valine and leucine side chains was also obtained. Difficulties encountered in the observation of ^{13}C spectra using superconducting solenoids will be discussed.

F-POS-E8 ^{1}H and ^{31}P NMR STUDIES OF THE PROPIONYL COENZYME A CONFORMATION ON TRANSCARBOXYLASE. C.H. Fung, R.J. Feldmann* and A.S. Mildvan, Institute for Cancer Research, Philadelphia, Pa. 19111 and Division of Computer Research, NIH, Bethesda, Md. 20014

The effects of three preparations of transcarboxylase, containing varying mole ratios of Zn(II), Co(II), and Cu(II), at the 12 metal sites, on the longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates of 12 protons and the 3 phosphorus atoms of the bound propionyl CoA, were measured at 100 and 40.5 MHz. The paramagnetic contributions of the tightly bound Co(II) at the active site, to $1/T_1$ of these nuclei were obtained by solving the appropriate simultaneous equations. The resulting $1/T_1$ values and the correlation time (2.2 psec) determined from the frequency dependence of $1/T_1$ yielded absolute distances (in Å) from the bound Co(II) to the following protons (A, Pa and Pr stand for adenine nucleotide, pantetheine, and propionyl moieties, respectively): AH (2), 6.8; AH(8), 6.9; AH(1'), 7.2; PaH(3), 8.2; $P_aH_2(9)$, 7.7; $P_rH_2(2)$, 6.5; and $P_rH_3(3)$, 8.7, as well as lower limit distances to 5 additional protons ($\geq$7.4 Å) and to the phosphorus atoms ($\geq$9.4 Å) of the bound propionyl CoA molecule. These 15 distances were used in a computer search among 47000 rotamers for that conformation of propionyl CoA which minimized van der Waals overlaps. The best fit structure shows a U shape about Co(II) with an anti adenine-ribose conformation. Since the estimated Co(II) to thioester distance is too great for direct coordination and since propionyl CoA does not change the paramagnetic effect of bound Co(II) on $1/T_1$ of water protons, the bound Co(II) does not directly activate propionyl CoA.



F-POS-E9 DEVELOPMENT OF SENSITIVE RECORDING SYSTEMS FOR ONE-MEGAVOLT ELECTRON MICROSCOPY OF BIOLOGICAL SPECIMENS.
Murray Vernon King and Donald F. Parsons, Electron Optics Laboratory, Roswell Park Memorial Institute, Buffalo, NY 14203.

High-voltage electron microscopy of biological specimens is currently hampered by the fact that the existing recording materials (photographic emulsions and phosphor screens) fall far short of the theoretical quantum limit in the sensitivity vs. resolution relationship at higher electron energies. This entails inordinately long exposures of specimens to the electron beam to focus and to record the image, with consequent excessive radiation damage. The limiting exposures for preservation of structural details of biological materials are in the range of 10^{-3} Coul cm^{-2}, while viability of living specimens can be maintained only at or below exposures of 10^{-5} Coul cm^{-2}. New systems under test are characterized on the basis of the sensitivity attained for a given resolution level, and accepted or rejected on this basis. Sensitivity is measured on photographic materials such as x-ray films, with or without intensifying screens (either standard x-ray screens or sheets of scintillating plastic), while resolution is estimated from the sharpness of shadow edges or of images of specimens projected onto films for which part of the field is in contact with a screen. Phosphor-fiber optics image-conversion plates designed for coupling to low light level television systems are evaluated both for yield of photons per electron as a function of electron energy and phosphor thickness and for resolution in the transmitted images. Selection of systems can be facilitated by Monte Carlo simulation of electron-beam spread in various materials. The authors thank Drs. Hans Ris and Dale E. Johnson for collaboration in the use of the high-voltage electron microscope at Madison, Wis. This work was supported by NIH Grant RR 00754-01.

F-POS-E10 TWO STAGE ANAPHASE, NUCLEAR MORPHOGENESIS AND INTERMICROTUBULAR STRUCTURES IN BARBULANYMPHA. Shinya Inoué and Hope Ritter*. Program in Biophys. Cytol., Dept. of Biol., Univ. of Penna., Philadelphia, Pa.; Dept. of Biol., Univ. of Georgia, Athens, Ga.; Mar. Biol. Labs. Woods Hole, Mass.

Utilizing Ritter's successful culture of symbiotic flagellates of the wood roach Cryptocercus punctulatus, we followed single Barbulanympha cells through division by combined polarized light and differential interference microscopy. In Barbulanympha, spindle is extra-nuclear and the nuclear envelope persists throughout mitosis. Kinetochores embedded in the nuclear envelope attach to astral rays and link chromosomes to spindle poles. I. Shortening of astral "chromosomal" fibers: draws the nucleus to the previously formed spindle, assists in spindle envelopment by the nucleus and pulls chromosome kinetochores to the stationary spindle poles (anaphase A). Subsequently the spindle elongates several fold and separates the chromosomes further (anaphase B). The separation of chromosomal fiber shortening and central spindle elongation in this two stage anaphase provides a unique opportunity for analyzing chromosome movement mechanisms. II. In early anaphase A, the lateral margins of the nucleus are pulled around the spindle by shortening astral rays, meet anteriad, and gradually form a seam parallel to the spindle axis. The seam of the resultant nuclear tube forms a cleft which opens as with parting lips at midpoint of the seam; the cleft enlarges perpendicular to and around the spindle to finally complete karyokinesis. Karyokinesis is thus accomplished through an intricate topological maneuver, or nuclear morphogenesis. III. A new method of image enhancement exposes periodic structures between microtubules in longitudinal section electron micrographs of anaphase A central spindles. These may be intertubular linkers or they may be dynein arms.

Supported by NSF Grant #GB-31739X and NIH Grant #5-R01-CA-10171-09.

F-POS-E11 PREPARATIVE FRACTIONATION OF AMPHOLYTES FOR ISOELECTRIC FOCUSING. J. C. Bagshaw, R. K. Brown*, J. M. Lull*, S. Lowenkron*, and S. N. Vinogradov . Wayne State University School of Medicine, Detroit, Mi 48201.

Narrow-range ampholytes for isoelectric focusing in polyacrylamide gels were prepared by fractionation on ion exchange resins. Broad-range ampholytes were synthesized by the copolymerization of acrylic acid with oligoethylene oligoamines (Vinogradov, et al., Biochem. Biophys. Res. Comm., 54, 501, 1973). Adsorption of acidic components on anion exchangers yielded ampholyte preparations which produced stable pH gradients in the range pH 6-9. Such preparations were superior to the parent broad-range ampholytes for isoelectric focusing of proteins such as hemoglobins. Similarly, ampholytes prepared by adsorption of basic components on cation exchangers produced gradients in the range pH 2.5-6. Composition of unfractionated and fractionated ampholytes was investigated by analytical separation on a cation exchange resin. At least 60-70 peaks were detected in broad range ampholytes. Narrow-range ampholytes comprised the expected sub-sets of peaks found in the parent preparation. (Supported by USPHS Grants HL 15793 and HL 14063 and NASA Contract No. NAS 8-29823).

F-POS-E12 EFFECTS OF MAGNESIUM ON THE INITIAL RATES OF ATP HYDROLYSIS CATALYZED BY HIGHLY-PURIFIED SHEEP KIDNEY Na^+,K^+-ATPase (E). Taitzer Wang* and George E. Lindenmayer, Depts. of Cell Biophysics and Medicine, Baylor College of Medicine, Houston, Tx. 77025.

Initial velocities of ATP hydrolysis were measured by an automated version of a modified Martin-Doty assay for Pi. Assays were carried out in imidazole buffer (80 mM, pH 7.12) at 22°C with ATP varied from 0.066-0.878 mM, Mg^{2+} varied from 0.02-8 mM, and K^+ varied from 0.125-10 mM. Data analysis was carried out using a non-linear regression program (BMD07R). In the presence of 60 mM Na^+, the data fit a model that had one substrate site per E plus an additional regulatory site for Mg^{2+} and two activation sites for K^+. The model predicted that product formation derives from four enzyme complexes: those with K^+ at one or both of its sites with or without Mg^{2+} at its regulatory site. Mg^{2+} had four other effects on rates of ATP hydrolysis. (1) Its chelate with ATP, (Mg·ATP), formed the true substrate for the reaction. (2) Mg^{2+}, through interaction with the regulatory site, conferred asymmetry on the two sites for K^+ with respect to affinity. (3) Mg^{2+}, through interaction with its regulatory site, modulated the antagonism between K^+ and (Mg·ATP). When the regulatory site was unoccupied by Mg^{2+}, K^+ could decrease the affinity of E for (Mg·ATP) by a factor of 600. When the regulatory site was occupied by Mg^{2+}, K^+ could decrease the affinity of E for (Mg·ATP) by a factor of only 3.5. (4) Excess Mg^{2+} inhibits activity of E in the presence of high K^+ concentrations.

F-POS-E13 A MULTIPLE-PATH FILTER TO STUDY THE BIOLOGICAL EFFECTS OF STRATOSPHERIC OZONE CHANGES: Ronald E. Davies and P.D. Forbes*, The Photobiology Program, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pa., 19140.

The possibility of technology-related changes in stratospheric ozone concentration has aroused considerable interest. A reduction in ozone concentration would reduce optical filtration and would result in increased transmission of solar ultraviolet light of less than 320nm wavelength; possible biological consequences are a source of speculation. While it is possible in theory to make reasonable estimates of the magnitude of the spectral changes resulting from given changes in ozone concentration, the further prediction of biological consequences requires knowledge of biological action spectra; few of these are well studied. In the absence of known action spectra, the effects of specific source spectrum changes can best be studied by simulating these changes as closely as possible. To simulate the effects of environmental ozone path changes, we have devised a multiple-path filter assembly in which the filter material is ozone. The system consists of a concentric series of three quartz cylinders surrounding a long-arc xenon lamp, with Teflon partitions to create three angular sectors. Selection of the diameter of the cylinders, and control of the gas flow path, establishes three effective paths differing by accurately known ratios. Ozone, produced by a commercial generator, is flowed through the inner cylinder, 2 sectors of the second cylinder, and one sector of the outer cylinder; the remaining sectors are purged with nitrogen. Ozone concentration and UV flux are monitored continuously. The system has been constructed and is being tested. (Supported in part by National Cancer Institute contract NO1CP43271).

F-POS-E14 ANALYTICAL CELL ELECTROPHORESIS IN A STATIONARY DENSITY GRADIENT. R.A. Gaines*, R.C. Boltz, Jr.*, and P. Todd, Department of Biophysics, The Pennsylvania State University, University Park, Pennsylvania 16802.

The concept that electrophoretic mobilities of biological cells can be determined by bulk electrophoresis in a stationary density gradient was tested. Erythrocytes from rat, chick, and rabbit were fixed in 2.5% glutaraldehyde and used as test particles. By the microscopic method of electrophoresis in low-conductivity (1.2 mmho/cm) buffer their electrophoretic mobilities were found to be -1.19 ± 0.08, -1.07 ± 0.11, and -0.72 ± 0.10 cm-μm/V-sec, respectively. These same test particles were studied by measuring their velocities during vertical upward electrophoresis of the three-component cell mixture in a cooled glass column 2.2 cm in diameter through an isotonic sucrose-ficoll gradient. Three sharp bands of migrating cells were visible within 15 min of application of an electric field of 13.4 V/cm. The bands remained stable and clearly resolved during 2.0 hr of constant-field electrophoresis. The identification of the correct cell bands was accomplished by harvesting the gradient and determining that the middle band contained the nucleated chick erythrocytes. These studies suggest that density-gradient electrophoresis is a valid method for separating cells on the basis of charge and for exploring their surface properties.

Work supported by grant No. 5 R01 CA 12589-03 and contract No. NO1-CB-43984 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

F-POS-E15 A TECHNIQUE FOR EVALUATING BLOOD PERFUSION RATE IN MUSCLE AND VISCERAL ORGANS. R. M. Roppel and E. W. Retzlaff, Department of Biomechanics, Michigan State University, East Lansing, Michigan 48824.

In efforts to develop a means for evaluating relative rates of blood perfusion through solid tissues, microbead thermistors of special construction were implanted via small hypodermic needles in muscle and visceral organs of hamsters. Both normal animals and a strain bearing hereditary muscular dystrophy were used, the general purpose being to test the hypothesis that the muscle disease is causally related to a defect in vascular control mechanisms. The thermistors were powered by a constant-current source to operate in the self-heating mode, the voltage drop across the thermistor being taken as a measure of blood perfusion rate in the vicinity of the bead. This potential was recorded as a function of time, while the instrumented hamsters were administered a variety of naturally-occurring and synthetic vasoactive substances. Results of these studies are described and the potential of this kind of probe as a diagnostic tool is discussed. The assistance of Mr. Norman St. Pierre is gratefully acknowledged. (Supported in part by the Muscular Dystrophy Association of America.)

F-POS-E16 MANGANESE AND OXYGEN EVOLUTION IN RECONSTITUTED CHLOROPLASTS. R.E. Blankenship*, G.T. Babcock, and K. Sauer, Dept. of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, California, 94720.

The probable participation of Mn in the mechanism of O_2 evolution from chloroplasts was reinforced by the recent report that inactivation using tris washing or similar procedures produces an EPR spectrum characteristic of aqueous manganous ion. The evidence supported a view that the manganese is released from a membrane-bound site into the interior aqueous space of the closed thylakoid membrane sacs [Blankenship and Sauer, BBA 357 (1974) 252-266]. We have now investigated the properties of tris-washed chloroplasts that have been reconstituted by removal of the tris in the presence of reducing agents. O_2 evolution was restored fully to the control level. Furthermore, the characteristic induction, the period four response to a series of 10 μsec light flashes and the DCMU sensitivity were again observed in the reconstituted chloroplasts. At the same time, the large EPR signal of aqueous Mn^{2+} decreased to a very low level, despite the fact that the reconstituted membranes still contained 65% of the control level of Mn. We interpret the results to suggest that two-thirds of the chloroplast Mn is normally incorporated into a membrane-bound site that is close to the inner thylakoid surface and is EPR-silent. The presence of Mn in this membrane site correlates with the ability of the thylakoids to evolve O_2 from water under illumination.

F-POS-E17 EPR DETECTION OF THE PHYSIOLOGICAL DONOR TO $P680^+$ IN OXYGEN-EVOLVING CHLOROPLASTS AT ROOM TEMPERATURE. G. T. Babcock, R. E. Blankenship*, J. T. Warden and K. Sauer, Department of Chemistry and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

In illuminated, oxygen-evolving spinach chloroplasts at room temperature, a free radical transient is observed which has kinetic characteristics similar to those predicted for $Z^{+\bullet}$, the physiological donor to the Photosystem II reaction center chlorophyll. Following a 10 μsec light flash, this species is fully formed within the 100 μsec response time for our detection system. The subsequent decay occurs via a first order process with a halftime of approximately 700 μsec. An analogous signal is detected in whole cells of the green alga, Chlorella pyrenoidosa. The EPR spectrum for this transient in the $g \approx 2.00$ region is similar to that observed for chloroplast EPR Signal II. DCMU inhibits the formation of this species under conditions in which it inhibits the formation of $Z^{+\bullet}$. Following inhibition of oxygen evolution by treatments which act on the water side of Photosystem II, the decay time for this radical is increased to approximately 1 sec. The addition of exogenous, lipophilic electron donors (e.g., phenylenediamine, benzidine, diphenylcarbazide) to restore electron flow through Photosystem II decreases the radical decay time to an extent determined by the donor concentration.

F-POS-E18 TECHNIQUE FOR MEASUREMENT OF IN VIVO CHANGES OF NADH LEVELS IN CANINE MYOCARDIUM BY SURFACE FLUOROMETRY. C.W. Goodwin*, C.H. Barlow*, A. Mayevsky*, L. Mela* and B. Chance, (Intr. by R.W. Woody), Johnson Foundation, University of Pennsylvania, Philadelphia, Pa. 19174.

Changes in mitochondrial redox states in the myocardium of living animals in response to altered oxygen availability may be conveniently measured by monitoring changes in NADH fluorescence. The custom built D.C.-fluorometer utilized two photomultipliers and a three branched light pipe connected as follows: 45% fibers - 366 nm exitation source, 45% fibers - photomultiplier with 460 nm filter, 10% fibers - photomultiplier with 366 nm filter. The common end of the light pipe (4 mm diameter) containing the randomly mixed fibers from the three branches was secured gently to the surface of the myocardium of an anesthetized dog by a specially designed cannula. Responses of NADH fluorescence to graded hypoxia and ischemia in control and hemodiluted dogs were measured. Typically the control dogs showed an increased NADH fluorescence and decreased reflectance only when a threshold level of 5% O_2 in the inhalation mixture was reached. Lower O_2 concentrations produced correspondingly higher NADH reductions and reflectance changes. Hemodiluted dogs reached a threshold at higher inspired O_2 levels and showed markedly reduced reflectance changes. Ischemia, produced by occlusion of the coronary artery, resulted in a more rapid and considerably larger increase in NADH fluorescence with proportionately smaller reflectance changes. This technique thus offers a sensitive method for measuring changes in intracellular redox states in the working myocardium of intact animals and for evaluating potential therapeutic interventions. Supported by USPHS Grants GM01540, HL02396, GM19867 and HL15835.

F-POS-E19 COLLAGEN FIBER ORIENTATION IN VARIOUS COMPONENTS OF HUMAN CORTICAL BONE‡. P. Frasca*†, Division of Orthopaedic Surgery, Albany Medical College, Albany, N.Y. 12203; and R.A. Harper* and J.L. Katz, Laboratory for Crystallographic Biophysics, Department of Physics, Rensselaer Polytechnic Institute, Troy, N.Y. 12181.

Techniques recently developed in our Laboratory for isolating single osteons and for separating the lamellae of all lamellar bone structures, subsequent to decalcification, have enabled studies of the orientation of the collagen fibers and fibrils within their lamellae. These studies employ scanning electron microscopy which allows the collagen fibers and fibrils to be viewed directly. Human bone sections removed from adult femur and from young tibia were obtained shortly following amputation. Cross sections 100-300μ thick were sliced on a low speed diamond wheel cutter keeping the sample wet with distilled water. Thin sections were decalcified by immersing them in a solution of 0.5M EDTA of pH=7.4 at room temperature for 3 hours or at 4°C for a week after which time the sample was shown by x-ray diffraction to be completely decalcified. The decalcified sections were then observed under polarized light and osteons of known type as well as pieces of circumferential and endosteal bone were dissected out of the thin sections. The osteon samples were kept wet during manipulation in order to maintain the compliancy of the collagenous structure and thus prevent the sample from cracking. Each sample was mounted on a standard SEM aluminum stub by means of silver paint and coated under a vacuum evaporator with a 60-40 Au-Pa alloy. The samples were observed with a MAC Model 700 scanning electron microscope at an acceleration voltage of 20kV.

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†NIDR Special Postdoctoral Fellow.

F-POS-E20 ISOLATION OF CELL SUBPOPULATIONS FROM IN VITRO TUMOR MODELS BY SEDIMENTATION AT UNIT GRAVITY.[1] R.E. Durand, Radiobiology Research Laboratories, Department of Radiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

Chinese hamster V79-171 cells grown in vitro as asynchronous single cells, as plateau-phase cultures, or as multicell spheroids, contain subpopulations that differ in cell volume according to their position in the cell cycle. These subpopulations can be isolated by sedimentation at unit gravity. Asynchronous cells were found to sediment with a modal velocity of 17.7 mm/hr, whereas the modal sedimentation velocity of plateau-phase and spheroid cells was found to be 16.0 mm/hr and 15.1 mm/hr respectively. The composition of the various subpopulations of cells was monitored using either tritiated thymidine incorporation or sensitivity to gamma radiation as an index of cell cycle position. The plateau-phase and spheroid cultures were found to contain larger numbers of G_1-like populations. In addition, the G_1-like cells from spheroids sedimented much slower, suggesting that prolongation of this noncycling state may lead to additional decreases in cell volume or density. However, no change in viability, that is, growth potential, of these cells was observed. Since these sedimentation velocity techniques permit isolation of functionally different cell subpopulations after treatment with cytotoxic agents, it now will be possible to assay differential lethality when such agents are applied to cells growing in situ in a tumor-like situation.

[1]Supported by ACS Institutional Grant IN-35M and NIH Center Grant CA-06295.

F-POS-E21 MEASUREMENT OF H/e RATIOS IN SPINACH CHLOROPLAST USING FLASHING LIGHT. Charles F. Fowler, Department of Bioscience, Martin Marietta Laboratories (RIAS), Baltimore, Maryland 21227.

A sensitive and rapidly responding pH electrode has been used to measure H/e ratios during single turnovers of the electron transport system. The pH changes induced by single flashes were measured and compared using methylviologen (ΔpH_m) and ferricyanide (ΔpH_f) as Hill acceptors. With both acceptors the first few flashes given after a dark period induced proton uptake with a rise time of ~30 msec followed by a slow (> 1 sec) recovery. The ratio $\Delta pH_m / \Delta pH_f$ induced by these early flashes was 2 over a wide pH range. After several flashes, a constant pH level was reached in methylviologen. When illumination ceased the pH reverted back to the original baseline. In contrast, with ferricyanide as the acceptor, once the steady state was reached, a constant net acidification occurred after each flash. This increment (ΔpHe) is a measure of electron transport (1 H^+ per flash). The ratio of proton uptake to electron transport in ferricyanide ($\Delta pHf / \Delta pHe$) was 2. In methylviologen this ratio ($\Delta pH_m / \Delta pHe$) was 4.

These data imply that for each electron moved through the photosynthetic chain 3 protons are released inside the thylakoid with ferricyanide as an acceptor and 4 with methylviologen.

The occurrence of 4 loci for proton accumulation is in good agreement with the chemiosmotic hypothesis in view of recent ATP/2e and H/ATP determinations.

F-POS-E22 MITOCHONDRIAL CYTOCHROME c OXIDASE: FUNCTION AND PROPERTIES OF THE "INVISIBLE" COPPER. J. Gordon Lindsay, David F. Wilson and Charles S. Owen, Johnson Research Foundation, Dept. of Biophys. and Phys. Biochem. Univ. of Pennsylvania, Philadelphia, Pa. 19174.

Cytochrome c oxidase contains two cytochromes (a and a_3) and two copper atoms but only one of the copper atoms has been shown to contribute measurable optical and epr absorption properties while the other has remained "invisible". Carbon monoxide forms a readily measurable compound with reduced cytochrome c oxidase (α max 590 nm; Soret max 430 nm) and a dissociation constant of 0.6 μM. Measurements of the oxidation-reduction potential dependence for formation of this compound in isolated oxidase, mitochondria and submitochondrial particles give an n value of 2.0 at high CO concentrations (> 10 μM) with deviation toward n=1 at lower CO concentrations. These data provide evidence that CO binds with high affinity to a reduced cytochrome a_3-reduced "invisible" copper complex and not to either the reduced cyt. a_3-oxidized copper complex or the oxidized cyt. a_3-reduced copper complex. (Lindsay and Wilson, FEBS Letter, 48, 45-49). Competition between CO and oxygen suggests that oxygen reacts with the reduced cyt. a_3 reduced copper complex to form a bridged compound which is reduced in a 2e step to a stable bridged peroxide compound. This is then further reduced to water. Analysis of the titration curves at various pH values gives a pH independent E_m of 340 mV and an n value of 1.0 for the "invisible" copper. Optical measurements at pH 8.3 (E_m a_3 = 290 mV) in submitochondrial particles show no measurable absorption changes associated with this copper except in the Soret region where a broad absorbance change near 420 nm titrates with an E_m of 345 mV. Supported by NSF grant GB 28125 and NIH grant GM12202.

F-POS-E23 LIGHT-INDUCED TURNOVER OF CHLOROPLAST CYTOCHROME b-559 AT LOW pH. P. Horton* and W.A. Cramer (Spon. R.A. Dilley) Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

To ascertain the function of chloroplast cytochrome b-559, it is necessary to see its turnover under physiological conditions. The proportion of cyt b-559 oxidisable during illumination by low intensity 732 nm light increases as the pH is decreased below 6.5. At pH 5.0-5.5 total oxidation is seen and subsequent red light causes reduction. The far-red oxidation at low pH is inhibited by DBMIB. Incubation of chloroplasts in lightly buffered medium at pH 5.0 can also cause dark auto-oxidation of cyt b-559 and subsequent addition of NaOH to raise the pH can bring about reduction. The auto-oxidisable nature of cyt b-559 at pH 5.0 suggests that at this pH it may exist in a lower potential form than at higher pH. Far-red induced oxidation also occurs at higher pH after pre-treatment of chloroplasts with high intensity light. The degree of 'light activation' is pH dependent, being more pronounced at lower pH; after light activation total cyt b-559 oxidation by far-red light can now occur up to pH 6.0. It is suggested that during physiological illumination conditions the change in internal pH could allow cyt b-559 to function in the main electron transport chain. Supported by NSF grant GB-34169X.

F-POS-E24 INTRAMEMBRANE LOCATION OF MITOCHONDRIAL CYTOCHROME HEMES. George D. Case and John S. Leigh, Jr., Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania, 19174.

EPR Spectra of the ferricytochromes were studied in normal and nickel-plated pigeon heart mitochondria (PHM) and submitochondrial particles (PHSMP). Whenever Ni^{2+} can approach to within 10Å of a heme group, acceleration of the heme EPR relaxation rate should elicit observable shifts in the maxima of power saturation curves, and also less detectable lineshape changes. In PHM, at least 3 peaks attributable in part to cytochromes c_1, b_K, and b_T are observed at g=3.3,3.4$^+$, and 3.7. All of these are sensitive to Ni^{2+}. Ni^{2+} has no effect on the g=3.3 peak (c_1 and b_K) in inverted PHSMP. In PHSMP, the g=3.4$^+$ peak is missing, and the g=3.7 peak (b_T) resolves into two species, neither of which responds to Ni^{2+}. Consequently, both cytochromes b_K and b_T, as well as c_1, lie very near the exterior surface of the inner mitochondrial membrane. Cytochrome oxidase saturation curves were obtained for both the high-spin (g=6) and low-spin (g=3.1)forms. Neither signal reveals any influence of Ni^{2+} either in PHM or PHSMP. Hence, the oxidase hemes are buried in the membrane away from H_2O. Ni^{2+} also fails to perturb the cytochrome c saturation curve in PHM or PHSMP, or even in free solution except with a great excess of Ni^{2+}. Evidently, the protein sphere is large enough to hide the heme. The present observations are not inconsistent with the results which other investigators have obtained using other techniques, since the accessibility of a protein portion of a cytochrome to H_2O may differ from that for its heme groups. This work supported by USPHS grant GM 12202 and NSF grant BMS74-08132.

F-POS-E25 PARTICLE SIZE DISTRIBUTIONS OF HUMAN PLASMA LIPOPROTEINS BY INTENSITY CORRELATION SPECTROSCOPY. C.B. Bargeron*, R.L. McCally* and M.H. Friedman, The Johns Hopkins University Applied Physics Laboratory, Silver Spring, Maryland 20910 and S. Margolis*, The Johns Hopkins Medical School, Baltimore, Maryland 21205.

The technique of intensity correlation spectroscopy has been applied to the study of particle size distributions of human plasma lipoproteins. Laser light scattered from the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) fractions was detected by a photomultiplier; the resultant photocurrent was autocorrelated. The correlation spectra were analyzed by the method of cumulants. The cumulants reflect the distribution of diffusion constants of the scatterers. This distribution of diffusion constants is in turn related to the distribution of particle sizes by the Einstein-Stokes equation. The quantitative accuracy of this technique has been demonstrated using a suspension of polydisperse polystyrene spheres.[1] In addition to providing an appropriate means of investigating the natural polydispersity of plasma lipoproteins under equilibrium conditions in solution, these techniques are also being applied to study aggregation reactions of LDL.

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[1] C.B. Bargeron, J. Chem. Phys. 61, 2134-2138 (1974).