the AMA. Each of these plans address health care coverage, plan financing, and cost containment strategies to varying degrees, yet none of the plans specifically address the role of technology, other than to suggest that technology assessment would be required for success of the plan (PNHP and CCHP). However, implementation of any of the plans would have direct and/or indirect effects on the availability, use, and development of medical technology, and hence, the quality and cost of health care under the plan.

91-18. The Oregon Priorities: A Bioengineering Resource

J.D. Andrade and R. Huefner, Departments of Bioengineering and Political Science, The University of Utah

The State of Oregon is leading the nation with an ambitious and important effort to address the health care cost problem (J. Kitzhaber, "A Healthier Approach to Health Care," *Issues in Science & Technology*, Winter 1990–91, pp. 59–65).[1] After a 2-year effort, involving all segments of the Oregon population, Oregonians have categorized and prioritized health care into 17 generic categories and nearly 800 specific procedures. At the top of their priority list are ". . . life-threatening conditions for which treatment will return a person to health; maternity services; preventive care for children such as screening and diagnosis; and preventive care for adults." At the bottom are ". . . treatments which will marginally improve a person's quality of life although they may not prolong it." (Oregon Basic Health Services Program Report, April, 1991, Dept. of Human Resources, Office of Medical Assistance Programs, State of Oregon, Salem, Oregon).[2] The data they have collected and the assessments and prioritization they have developed can serve as a resource for bioengineering researchers and developers in their choice of cost-effective projects and studies. We review the Oregon categories and suggest areas where academic bioengineering activities could have an important influence on decreasing the costs of health care.

II. CARDIOPULMONARY ENGINEERING

II.1. *Fluid Shifts in Humans Exposed to Microgravity*

Organizer: Alan R. Hargens, NASA Ames Research Center

91-19. Fluid Shifts in Humans with Actual and Simulated Microgravity—An Overview

Alan R. Hargens, Life Science Division (239-11), NASA-Ames Research Center

On Earth, gravity normally imposes blood pressure gradients on the cardiovascular system. These gradients increase blood pressure, blood flow and fluid accumulation in dependent tissues of the body. On the other hand, actual or simulated microgravity causes blood and tissue fluid to shift from the lower to upper body. Studies of humans in space have documented increased heart rate, narrowed pulse pressure, reduced plasma volume, decreased heart size, headache and facial edema. Return of astronauts to Earth is accompanied by orthostatic hypotension and decreased exercise capacity. These factors reduce performance during descent from orbit and increase risk during emergency egress from the space craft. Models of simulated microgravity that are relevant to the cardiovascular system include head-down tilt, water immersion, and prolonged horizontal bedrest. Further verification of ground-based models with flight experience is needed along with development of noninvasive technology in order to understand underlying mechanisms of adaptation. Possible countermeasures to speed readaptation of crew members to gravity after prolonged space flight include exercise, lower body negative pressure, and artificial gravity. (This research was supported by NASA.)

91-20. Cardiovascular Studies on SLS-1: The First Dedicated Life Science Spacelab Mission

Jay C. Buckey, University of Texas Southwestern Medical Center

Experiment 294 on SLS-1 (one of three cardiovascular experiments, P.I.: C. Gunnar Blomqvist) was designed to measure the human cardiovascular adaptation to zero-gravity and the

readaptation to one-G. After returning from space, astronauts have difficulty regulating their blood pressure, some to the point of fainting during quiet standing. Also, upright exercise capacity is reduced. The goal of the experiment was to provide both the magnitude of and mechanisms for the changes in blood pressure regulation. To accomplish this, several key cardiovascular variables had to be measured accurately (and non-invasively if possible) over time. Central venous pressure, heart rate, blood pressure, leg volume, leg flow, leg compliance, maximal exercise capacity, and cardiac size were measured pre-, in-, and post-flight. Several new devices and techniques were developed to provide the information including an ambulatory central venous pressure device, a pre-calibrated plethysmograph, and three-dimensional echocardiography.

91-21. Influence of Actual or Simulated Weightlessness on Human Arterial Baroreflex Function

D.L. Eckberg and J.M. Fritsch, Departments of Medicine and Physiology, Hunter Holmes McGuire Department of Veterans Affairs Medical Center and Medical College of Virginia

Astronauts exposed to microgravity for as little as ten days may experience unusual cardioacceleration and arterial pressure reduction when they stand after reentry. Although blood volume reduction may contribute to these changes, other factors may also participate. We tested the possibility that actual or simulated microgravity impairs arterial baroreflex function in several groups, including volunteers for ten and 30 day 6° head-down bedrest studies, and astronauts before and after four to ten day Space Shuttle missions. We studied vagally-mediated baroreceptor-cardiac reflex mechanisms by delivering stereotyped pressure changes to a neck chamber. Initially, pressure was raised to about 40 mmHg, to compress the carotid sinuses. Then, neck pressure was reduced by 15 mmHg decrements, to −65 mmHg, in synchrony with R-waves. We correlated changes of baroreflex responses with changes of hemodynamic responses to standing. Our results suggest that weightlessness alters baroreflex function in several ways. The baroreceptor stimulus-R-R interval response relation is shifted down on the R-R interval axis, and the range and maximum slope are reduced. These changes are associated with (and may contribute to) the reduced orthostatic tolerance that develops in humans exposed to microgravity.

91-22. Cerebral Blood Flow Changes in Microgravity

R.L. Bondar,[1] M.S. Kassam,[2] F. Stein,[2] P. Dunphy,[2] and R. Aaslid,[3] [1]Canadian Space Agency, Ottawa, [2]CATE, Ryerson Polytechnical Institute, Toronto, [3]Department of Neurosurgery, Inselspital, Berne

Spaceflight is not easily accessible for gathering repetitive data on physiological adaptation to microgravity. We have utilized brief periods of microgravity on NASA's KC-135 aircraft to evaluate mean blood flow velocities (MBFV) insonated from the right middle cerebral artery in 20 subjects by a 2 MHz transcranial Doppler (TCD) probe through the right temporal window, simultaneously with arterial blood pressure. Cardiac cycle by cardiac cycle analysis of digitized data was performed offline. Heart rate (HR) decreased upon entry into microgravity and increased in the 2g pullup. MBFV changes preceded those of mean arterial blood pressure (MABP), and were more useful than pulsatility indices in defining these dynamic events. The data did not return to baseline during any part of a parabola. Upstream parameters—HR, MABP—affect the nature of the TCD waveform. Cerebral autoregulation can be assessed by this concomitant simultaneous measurement of MABP and MBFV of the middle cerebral artery.

91-23. Countermeasures for Postflight Orthostatic Intolerance

R.A. Pinter, S.M. Fortney, KRUG Life Sciences and NASA Johnson Space Center

Postflight orthostatic intolerance has been a significant problem since the beginning of human space flight. Possible mechanisms for this intolerance include: (1) Loss of body fluids, (2) Changes in autonomic function, (3) Changes in vascular compliance and, (4) Altered endocrine function. Both mechanical and pharmacological countermeasures to this intolerance have been studied. Mechanical countermeasures include: (1) Anti-g suits, (2) In-flight exercises,

(3) Acceleration, (4) Lower body negative pressure, (5) Thigh occlusion cuffs, and, (6) Fluid loading solutions. Proposed pharmacological countermeasures include: (1) Sympathomimetics, (2) Mineralocorticoids, and (3) Vasopressin. The most successful therapeutic regimen, however, will probably include a combination of several of the above countermeasures.

91-24. Techniques for the Investigation of Central Hemodynamics under Conditions of Altered Gravitational Stress

Ricky D. Latham, Curtis D. White, and John W. Fanton, Lab for Aerospace Cardiovascular Research, Armstrong Laboratory

Much data has been recorded on the hydraulic performance of the heart and vascular system in supine man during invasive cardiac catheterization procedures. Little data exists, however, describing ventricular/vascular function in sophisticated hydraulic terms for the upright posture, which is the most clinically relevant to man. Since practical and ethical constraints preclude many of the necessary studies we have developed a chronically instrumented primate model for these investigations. Baboons are trained to accept the study environment, then instrumentation is placed surgically: Ao and LV pressure cells, Ao root flow, 3 axes of LV dimensions by piezoelectric ultrasound crystals, specialized vascular ports in the left and right atria, and an hydraulic occluder cuff. Study conditions include tilt in the physiologic lab (acute and 48-hour studies), acceleration for hypergravity, and parabolic flight with NASA's KC-135 for microgravity. We have had to modify the packaging of implanted pressure cells to ensure > 6-month viability. Custom-made kinkless tubing allows repeated interrogation of the heart by small (.035″ OD) high fidelity catheters. Several configurations of crystal types have been evaluated. We are developing a volumetric Doppler system more conducive to acceleration tests. We have engineering specialized jackets and confinement G-rated chairs for the study subjects. Details of these modifications will be presented and examples of use. Examples of types of investigative hemodynamics using this model will be shown.

91-25. Body Segment Circumference Measurements for Monitoring Fluid Shifts with Simulated Microgravity

D.E. Watenpaugh, NASA-Ames Research Center/Bionetics, Inc.

Blood and extravascular fluid move headward in humans existing in microgravity. This headward fluid shift may initiate the cardiovascular deconditioning observed in astronauts. Head-down tilt (HDT) at 6° purportedly simulates cardiovascular and other effects of microgravity, yet HDT has not been compared to microgravity under identical experimental conditions. We will compare HDT to acute microgravity exposure by measuring central and peripheral venous pressure changes and blood volume redistribution during parabolic flight. We require a convenient, non-invasive, sensitive means of assessing differences in acute cephalad fluid shifts between treatments. Ga/In-in-silastic strain gauge plethysmography is a standard method for measuring changes in leg volume (lower body fluid shifts). A pilot study was performed in 4 subjects to explore use of strain gauge neck volume changes to quantify fluid movements to and from the upper body. We found a 3.90% (±1.84, SE) increase in neck volume in supine position relative to standing ($p < 0.001$)). Elevating the legs 40 cm at the ankle (analogous to HDT) increased volume further to 4.75% (±1.87, SE), which was a significant increase from supine neck volume ($p < 0.005$). Percent changes in leg and neck volumes, therefore, should provide sensitive indicators of fluid shifts during comparison of HDT to actual microgravity in parabolic flight.

II.2. *Technological Innovations in Intravital Microscopy*

Organizer: Herbert H. Lipowsky, The Pennsylvania State University

91-26. Interstitial Distribution of Protein and Water by UV/IR Absorption Microscopy

Julius J. Friedman, Physiology/Biophysics, Indiana University School of Medicine

Diffusive protein transport depends on the concentration gradient between plasma and pericapillary fluid ($C_p - C_{pc}$). Generally lymph protein concentration (C_L) is used to estimate

C_{pc} assuming that protein in the interstitial space is uniformly distributed. However, the interstitial matrix consists primarily of a collagen and hyaluronic acid gel which acts as a sieve. Convective transport of protein should establish a gradient across the sieve and C_L must be lower than C_{pc}. Collagen excludes both protein and water whereas hyaluronic acid only effectively excludes protein. Thus by identifying the changes in the distribution of protein and water following tissue treatment with either collagenase and/or hyaluronidase, the distribution of the collagen and hyaluronic acid in the interstitial space can be defined and their contribution to the establishment of interstitial protein concentration gradient evaluated. Because aromatic amines exhibit significant absorption at 280 nm and essentially zero at 320 nm and water absorption at 1500 nm is significantly greater than that at 1000 nm, the extinction due to both protein and water can be estimated with the Beer-Lambert relationship. Preliminary data verify the presence of an interstitial protein concentration gradient the major part of which is due to the sieving and exclusion properties of both collagen and hyaluronic acid.

91-27. Biochemical Microscopy

B.J. Barber, Center for Biomedical Engineering, University of Kentucky

The objective was to determine the distribution of water, collagen and plasma proteins in the perimicrovascular space and distal interstitial matrix. Intravital video microspectrophotometry (Am. J. Physiol. 258: H556-564, 1990) was performed using wavelengths of 280, 320, 705, 1050 and 2000 nm. The images were analyzed to give water, protein, and collagen spatial distributions in vascular and avascular regions of rat mesenteric tissue. Perimicrovascular protein concentrations were fitted to an exponential decay model; the decay constant was: arterioles $11 \pm 3\ \mu m^{-1}$, exchange vessels 25 ± 25, and venules 57 ± 25. Average perimicrovascular protein concentration were: venules, 3.7 ± 1.6 g/dL; arterioles, 2.2 ± 0.2 g/dL; exchange microvessels 2.4 ± 0.3 g/dL. Fourier spectral analysis showed periodicities in longitudinal perivascular and ECM protein spatial distribution. Volume expansion produced changes in protein and water distribution. It is concluded that both longitudinal and radial gradients in protein distribution exist in the peri-microvascular space. A diffusion aggregation fractal model for protein transport may account for protein clumping. Supported by NIH AG10257 and THRI.

91-28. Assessment of Fluid Movement in Tissues by Fluorescence Photobleaching

R.J. Stock and R.K. Jain, Department of Chemical Engineering, Carnegie Mellon University

Fluorescence recovery after photobleaching (FRAP) can be used to measure diffusion and convective transport *in vivo*. In this technique a region of tissue containing uniformly distributed, fluorescently tagged macromolecules is briefly irradiated using a laser. This creates an area of low fluorescence (photobleach). By monitoring the fluorescence recovery of the photobleach the local diffusion coefficients and convective velocities may be determined. We have applied this technique to measure transport of FITC-BSA in normal and neoplastic tissues in the rabbit ear chamber (Proc. Natl. Acad. Sci. USA. 86:5385-5389, 1989). Currently, we are measuring fluid movements within interstitial tissue in response to changes in systemic blood pressure and osmolarity due to blood loss and injection of a hyperosmotic solution (7.5% NaCl/6% Dextran 70), respectively. Initial results show marked absorption of fluid into the blood vessels after the systemic changes, especially following administration of the hyperosmotic solution.

91-29. Analytic and Engineering Foundations of 3-D Optical Sectioning Microscopy

W. Carrington, Program in Molecular Medicine, University of Massachusetts Medical School

3-D information can be obtained from a conventional light microscope by optical sections, that is by acquiring a sequence of 2-D images and changing the plane of focus for each image. The resulting 3-D images are unsatisfactory in several respects. The non-isotropic 3-D blurring distorts the 3-D shape of objects and out of focus light reduces contrast, obscures details and causes large errors in quantitative fluorescence measurements. The optical system is a linear system that can be characterized by its point spread which is measured by imaging a small flu-

orescent bead in 3-D. We combine this point spread function with optically sectioned images to reverse the optical blurring using an iterative deconvolution algorithm. The resulting 3-D image has improved resolution and substantial reduction of out of focus light. This approach has had considerable success in high resolution fluorescent imaging of isolated single cells. We discuss the possibilities of applying this approach to 3-D intravital imaging. We compare this approach to confocal microscopy, which rejects the out of focus light optically. This deconvolution approach may also be applied to 3-D confocal images with a corresponding improvement in resolution. We will discuss the optical aberrations that are present when focussing into a thick sample, their effect on image quality and methods for improving image quality. We will also discuss the computer hardware and software requirements for the deconvolution, display and analysis of 3-D images. (Supp NSF DIR-8720188.)

91-30. Application of 3D Optical Sectioning Microscopy to the Study of Microvascular Biology

G.A. Meininger, D.C. Zawieja, and Q. Li, Microcirculation Research Institute and Department of Medical Physiology, College of Medicine, Texas A&M University

The microvasculature is characterized by a highly-organized three-dimensional (3D) array of interconnecting blood vessels. Studies of the microcirculation using conventional intravital microscopy techniques provide two-dimensional (2D) views of its architecture. These 2D views lack information about the axial dimension and therefore underrepresent the spatial relationships that exist between vessels and their parenchymal environment and between different classes of vessels. 3D information would also be an invaluable adjunct to studies of events occurring at the level of vascular wall. Here a lack of differentiation in the axial dimension can interfere with elucidation of mechanical events and complex cell-to-cell or cell-matrix relationships. With the advent of optical sectioning techniques it is now possible to obtain a series of images with each representing a different focal depth. This is achieved by using a digital imaging microscope equipped with computer control of the focus and the camera readout for image acquisition. High resolution images are obtained with a cooled-CCD camera, digitized and stored for computer processing and 3D reconstruction. A graphics workstation is used to manipulate the 3D data set to display various views of interest and for feature extraction and analysis. With computerized control of the microscope stage the optical sectioning can be combined with automated orthogonal sampling. This permits collection of a series of spatially-related images from a single orthogonal image plane. These images can then be combined into a single montage view of a large area. In summary, these imaging techniques promise to be powerful new tools for the study of the microcirculation. (Supp HL33324 & AHA-Est. Invest. Awd. to G.A.M.)

II.3. *Transport Phenomena*

Organizer: Aleksander S. Popel, The Johns Hopkins University

91-31. Diffusion-Limited Reaction Between Membrane-Associated Proteins

Daniel A. Hammer, Stuart J. Bussell, and Donald L. Koch, School of Chemical Engineering, Cornell University

Reaction between membrane proteins is important in many cellular physiological responses, such as signal transduction in cellular growth control and electron transfer in oxidative phosphorylation. Importantly, many reactions are transport or diffusion limited, where the time for encounter between potential reaction partners is large compared to the time of reaction. Therefore, it is of interest to quantify the diffusion-limited reaction rate between membrane associated proteins.

We present results from several calculations to further elucidate diffusion-limited reaction rates. First, we have assessed the role of interparticle forces on the rate of encounter of two

freely diffusing proteins in a membrane and find the collision rate is greatly affected when the energy of interaction is as small as the thermal energy. Second, we have determined how the motion of a single protein is hydrodynamically coupled to the motion of a second, through calculation of the diffusion tensor for two cylinders embedded in a plane sheet of high viscosity relative to the surrounding aqueous solution; the time to capture between proteins is then calculated based on this spatially-varying diffusivity. Third, we have calculated the collision rate between two proteins when one is bound to a membrane, and the other can either diffuse along the membrane surface or diffuse in the aqueous space above the membrane and can transiently absorb or desorb with the membrane. This latter calculation is compared to data on cytochrome c, which reacts with membrane bound complexes on the inner mitochondrial membrane, and whose absorption equilibrium with the inner membrane depends on ionic strength.

91-32. Development of a Microcirculatory-Based Model of Sinusoid–Hepatocyte Exchange of Macromolecules

E. V. Cilento, R.L. Page, and A. Dasgupta, Chemical Engineering Department, West Virginia University

The liver is a diverse and complex organ that is responsible for removal and biotransformation of many solutes and toxins. It has a rich, anastomosing microvasculature providing intimate contact between the bloodstream and hepatocytes responsible for bio-processing of solutes. The value of a sinusoid-hepatocyte permeability coefficient (P_s) for 66 kD FITC-dextrans, calculated using video recordings of this fluorescent tracer movement through the hepatic microvasculature, was used with morphometric liver data available in the literature to develop an anatomically-based, four compartment model to describe biliary elimination of this solute by inert fluid-phase endocytosis. The model best fit available experimental bile outflow data for 70 kD FITC-dextrans when 95% of the solute taken up by hepatocytes moved through a slow vesicle compartment. This percentage is consistent with the intracellular mechanisms thought to be involved in endocytosis. The only other adjustable parameter was the hepatocyte-bile P coefficient which was determined to be 3.5×10^{-8} cm/s; a value consistent with P measured for continuous capillaries. The values available for the sinusoid P_sA_s product could not reproduce the experimental data unless reduced by a factor of 100; probably due to the inability of the model to account for the endocytic (controlling) mechanisms for biliary elimination. Further refinement of this model, which includes the transport phenomena involved in processing of solute via the slow vesicle pathway, will provide a useful microcirculatory model for elimination of macromolecules by endocytosis.

91-33. Transport of Fluid and Macromolecules in Tumors

Lawrence T. Baxter and Rakesh K. Jain, Harvard Medical School

In the treatment of solid tumors with therapeutic agents such as monoclonal antibodies the importance of physiological transport barriers is becoming more evident. Although able to bind selectively to cancer cells when tested in vitro, monoclonal antibodies have shown very limited success when tested clinically. For most tumors the uptake of antibodies and other macromolecules is inadequate and nonuniform.

We have recently developed a general theoretical framework for fluid and macromolecular transport in solid tumors (Baxter, L.T., and Jain, R.K., Microvascular Research 37:77–104, 1989). Our model predicts elevated and nonuniform interstitial pressure in tumors, and uses this information to estimate concentration profiles within tumors.

We have used a micropipette technique to test the model by measuring interstitial pressure in rats. For the first time the spatial distribution of interstitial pressure was determined quantitatively. In both a.c. and isolated tumor preparations a steep pressure gradient was seen at the tumor periphery.

These results have important implications for the use of novel macromolecular agents in tumor detection and treatment. The use of bifunctional antibodies, antibody-enhanced prodrugs, and other strategies for improving delivery will be emphasized.

91-34. A New View of Macromolecular Transport in the Arterial Intima

S. Weinbaum, Department of Mechanical Engineering, The City College of the City University of New York

A new conceptual view and theoretical framework will be presented for analyzing the filtration and macromolecular convective-diffusive transport processes in the intimal region of an artery wall. As proposed in the leaky junction-cell turnover hypothesis of Weinbaum et al. (1985) and experimentally confirmed in Lin et al (1988), macromolecules are assumed to enter the intima through a small fraction of transiently open junctions. In contrast to existing convective-diffusive models, which assume that the transport is primarily in a direction normal to the endothelial surface of the intima, the present model considers for the first time the nonuniform subendothelial pressure field that arises from the different hydraulic resistances of normal and leaky endothelial clefts and the special role that the internal elastic lamina (IEL) plays in modulating the horizontal transport of macromolecules after they have passed through the leaky clefts. The new theory is able to quantitatively explain the growing body of recent experiments in which an unexpectedly rapid early-time growth of the leakage spot has been observed and the longer time asymptotic behavior in which the leakage spot appears to approach an equilibrium diameter. The longer time model predictions are used to explain the time scale for the formation of lipid liposomes in subendothelial tissue matrix in animal feeding experiments where it has been observed that the extracellular lipid concentration rises prior to the entry of monocytes in the intima (Schwenke and Carew 1989).

91-35. Transcapillary Water and Solute Transport: Influences of Size, Charge, and Plasma Proteins

V.H. Huxley, Department of Physiology, University of Missouri

We find that the capillary permeability to solutes differs in plasma-perfused vessels from those perfused with albumin. Indirect evidence suggests an equivalent effect of plasma and albumin on water movement. These behaviors would occur if plasma, and not albumin, confers a transcapillary charge selectivity. This hypothesis was assessed by making paired measures of: (1) hydraulic conductivity (L_p) or (2) permeability (P_d) to an anionic solute, α-lactalbumin, or a cationic solute, ribonuclease A, in *in situ* frog mesenteric capillaries during exposure to plasma and albumin. Perfusion with plasma followed by albumin (BSA) did not alter L_p ($L_p^{Plasma}/L_p^{BSA} = 1.2 \pm 0.2$ (MEAN $\pm$ SEM); $n = 18$. Barrier resistance to α-lactalbumin was increased by plasma $(P_d^{Plasma}/P_d^{BSA})^{\alpha\text{-lactalbumin}} = 0.3 \pm 0.1$; $n = 10$. By contrast, plasma decreased barrier resistance to ribonuclease $(P_d^{Plasma}/P_d^{BSA})^{ribonuclease} = 2.4 \pm 0.4$; $n = 5$). Both an equivalent effect on water movement, and a greater resistance to an anionic solute are consistent with a negatively charged barrier which is absent during, or is removed by, albumin perfusion. (NIH HL 34872 and AHA EI.)

91-36. Oxygen Transport Issues in Photodynamic Therapy for Cancer

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Photodynamic therapy is a cancer treatment in which a tumor is exposed to optical radiation after a photosensitizer is administered. The resulting photosensitized reactions produce singlet molecular oxygen, which then causes tumor cell damage or death. Although the effective dose is dependent on many factors, the availability of normal free oxygen in the tissue can be a major limitation. We have studied this problem both theoretically and experimentally. Basic oxygen transport theory leads to the conclusion that, for a given total radiative flux, a treatment with on-off cycles in radiation should be superior to a treatment with a constant flux, because the off-cycle allows the tissue to recharge with oxygen. Experimental studies on tumor growth delay in rats confirm this prediction (T.H. Foster, R.S. Murant, R.G. Bryant, R.S. Knox, S.L. Gibson, and R. Hilf, *Radiation Research*, in press, 1991). In the present work, we refine the oxygen transport theory in an attempt to come closer to an optimum treatment strat-

egy. We show that better treatment strategies can be devised provided we have more data on the tumor, such as the degree of vascularization, the concentration of the photosensitizer, and some relation between singlet oxygen concentration and cell damage.

91-37. Oxygen Transport in Resting Skeletal Muscle: Theory vs Experiment

A.S. Popel, Department of Biomedical Engineering, School of Medicine, The Johns Hopkins University

Mathematical models are formulated for oxygen transport between blood vessels and muscle tissue in terms of nonlinear partial differential equations describing oxygen diffusion, convection, and chemical reactions. The effect of oxygen convection by capillaries in the vicinity of arteriolar vessels is systematically investigated. Numerical results are obtained for oxygen flux from arterioles as a function of capillary velocity, blood hematocrit, capillary spacing, and other relevant physiological parameters. The results are compared with experimental data obtained by microspectrophotometric measurements of hemoglobin saturation in individual arterioles in the hamster cheek pouch retractor muscle, from which the oxygen flux can be evaluated (L. Kuo and R.N. Pittman, Am. J. Physiol. 1989, 1991). A significant discrepancy is found between the predictions and the data. Possible sources of the discrepancy are critically assessed, including the assumptions regarding the Krogh diffusion coefficient and the intravascular transport. New results are also obtained on intracapillary transport of oxygen: the effects of red blood cell shape and oxygen dissociation curve on the capillary mass transfer coefficient are investigated. (Supported by NIH grant HL 18292.)

II.4. *Characteristics of Active and Passive Pulsatile Blood Flow in the Microcirculation*

Organizer: Stephen H. Nellis, University of Wisconsin

91-38. Phasic Pressure, Flow, and Diameter in the Microcirculation of the Freely Beating Heart

Stephen Nellis, University of Wisconsin, Division of Cardiology, Department of Medicine

Measurements of hemodynamic parameters in the coronary microcirculation proves difficult because the microscope magnifies motion. Past measurements have been made in either chemically arrested hearts or hearts which were strictly mechanically held. Continual developments have allowed the heart more freedom of motion. Now vessel pressure measurements can be made with only moderate restraint while vessel diameter and flow measurements can be accomplished with virtually no restraints. These measurements have revealed an exceptional phasic component in vessel diameter, pressure, and flow. This phasic component is larger than found in other tissues and unlike other tissues is most pronounced in venules. The large venous phasic component of 15 mm Hg is consistent between different vessels as well as different investigators. The venous diameter changes are large with average changes of 15% and are highly variable between vessels. They do not simply follow luminal pressures but instead could explain phasic pressures. Phasic changes in flow velocities are large with definite flow reversal in veins and probably flow reversals in arteries. The magnitude and pattern of phasic flow velocities are also variable between vessels but may depend on the preparation and the degree of restraint used to obtain the measurements.

91-39. Modelling of Coronary Pressure-Inflow Relationships

R.E. Mates, Departments of Mechanical and Aerospace Engineering and Medicine, University at Buffalo

Because the coronary circulation is subjected to contractile forces from the myocardium, inflow is dependent on cardiac contraction as well as on arterial pressure. Even during dias-

tole, an effective back pressure higher than right atrial pressure appears to limit inflow. The mechanism for this apparent "zero-flow pressure" remains controversial. Some investigators, including the author, have postulated that there exists in the microcirculation a point of pressure regulation, or vascular waterfall. Others have suggested that capacitance effects in the coronary circulation can explain the observed phenomena. In an attempt to differentiate between the two postulates, we have studied pressure-inflow relationships both in isolated perfused canine hearts and in intact anesthetised open chest dogs. We have studied these relationships in fibrillating hearts, during diastolic arrest, and in normally beating hearts at varying levels of vasomotor tone. When the coronary circulation is vasodilated with adenosine, step changes in pressure during a diastolic arrest produce step changes in flow with only a brief transient. We conclude that compliance effects on inflow in this circumstance are minimal. During normal beats, modelling of the pressure–flow relationship suggests that diastolic in-flow is very nearly equal to diastolic flow in the microcirculation. Thus, it appears that capacitance alone is not sufficient to explain observed pressure–flow relationships.

91-40. Pulsatile Blood Flow and Pressure in Skeletal Muscle Microcirculation

G.W. Schmid-Schönbein, S.-Y. Lee, and D.W. Sutton, Department of AMES-Bioengineering, University of California, San Diego

Although blood flow in the microcirculation has negligible inertia, time dependent pressure and flow variations are prominent. For example, a step arterial pressure at constant venous pressure leads to an arterial flow overshoot with a delayed venous flow which only in equilibrium approaches the arterial flow. In contrast, a step arterial flow is followed by a gradual rise of the arterial pressure, and sinusoidal arterial pressure and flow exhibit a nonlinear hysteresis. The venous pressures and flows are delayed in general compared to their arterial counterparts and there is a time dependent distribution of hemodynamic variables along the microvascular network. These phenomena arise from the interaction of the viscous stress in the blood and the stress in the viscoelastic walls of microvessels and has been designated as dynamic viscous flow (Biorheology, 26:215, 1989). Analysis shows that dynamic viscous flow is governed by an equation of the form:

$$\frac{\partial P}{\partial t} = C^2 \frac{\partial^2 P^3}{\partial z^2} \tag{1}$$

where P is the normalized blood pressure, t is time, z the axial coordinate along blood vessels, and C^2 is a coefficient which depends on the elastic distensibility of the blood vessel, the blood viscosity, and vascular geometry. In capillaries C^2 has low values, indicating rapid transmission of the arteriolar signal to the venous side, but in arterioles and venules C^2 is higher and the transmission is considerably slower. Solutions to Eq. 1 give qualitatively a realistic representation of the whole organ pressure-flow curves in resting skeletal muscles. Dynamic viscous flow in skeletal muscle has a number of implications for blood flow in skeletal muscle. It gives rise to the 'zero flow' pressure phenomenon during pressure pulsations, permits determination of the hemodynamic impedance in skeletal muscle for analysis of wave propagation, and serves to explain flow reversal during pressure pulsations at various hierarchies of microvessels. (Supported by a Grant from NSF DCB-88-19346.)

91-41. Effects of Muscle Contraction on Blood Flow

M.H. Laughlin, Department of Veterinary Biomedical Science and Medial Physiology, University of Missouri

Measurements of skeletal muscle blood flow in conscious animals performing locomotory exercise are considerably greater than blood flows measured in resting muscle during what is often called "maximal vasodilation." Indeed, the greatest blood flows measured in skeletal muscle (400 to 600 ml/min/100 g, depending upon species) have been measured during dynamic exercise in conscious subjects. The purpose of the experiments to be discussed was to determine why blood flows and apparent vascular conductance are greater during dynamic exercise than can be generated in vitro or in situ with various forms of artificial muscle activity. Blood flow

data obtained in resting skeletal muscle during maximal (pharmacological) vasodilation, will be compared to blood flow data obtained in various models of muscle activity. The data will be interpreted in light of the hypothesis that the muscle pump mechanism is a major determinant of perfusion of active skeletal muscle and that the efficacy of this pump is determined by the type of muscle contractions investigated. This analysis leads to the conclusion that skeletal muscle blood flow is determined by skeletal muscle vascular conductance, the perfusion pressure gradient and the efficacy of the muscle pump. (Supported by NIH grant #HL-36088 and HL-36531.)

91-42. Pulsatile Characteristics of Microvascular Blood Flow in Single Vessels

H.H. Lipowsky, Bioengineering Program, Penn State University

Numerous studies by direct intravital microscopy have measured the presence of substantial pulsatile components in intravascular pressures and red cell velocities in arterioles, capillaries and venules of the microcirculation. However, few studies have attempted to understand the significance of hemodynamic pulsatility in light of the non-Newtonian blood rheology and the viscoelastic characteristics of the blood vessel wall. To fill this void, studies of the microcirculation are conducted to measure intravascular pressures (P) and pressure gradients ($\Delta P/1$, dual-servo-null method) and RBC velocities (V_{rbc}, two-slit technique) in the mesentery of the cat. On line digitization of these parameters and simultaneous recordings of the EKG permitted signal averaging in concert with the r-r interval to obtain representative recordings of their pulsatile components. The results clearly demonstrate the presence of a nearly 90 degree phase shift between $\Delta P/1$ and V_{rbc} as red cells traverse the network from arterioles to venules, presumably due to an increase in microvessel compliance in postcapillary venules. To understand the effects of pulse pressure on the rheology of the microvessel wall, microocclusion experiments were performed to measure P at the proximal end of a vessel while occluding the distal end with a blunt probe. Analysis of the oscillating red cell column trapped within the a vessel from digitized video recordings revealed significant displacements of the vessel wall on the order of 0.01 to 0.05 μm. Preliminary data appear to support the conclusion of greater venule compliance compared to arterioles. The red cell column, however, appears to oscillate in a rigid body motion suggesting that compliance of the vessel wall is primarily responsible for the phasic relationships between pressure gradient and flow of the microvasculature. (Supported by USPHS NHLBI Research Grants HL-28381 and 39286.)

II.5. *Transport of Tracers, Metabolites, and Solutes in Cells and Organs: Mathematics, Methods, and Mapping*

Organizer: Thomas R. Harris, Vanderbilt University

91-43. Mapping of Endothelial Functions Between an In Vitro Cell-Column and In Vivo Studies

F.R. Haselton and J.S. Alexander, Biomedical Engineering, Vanderbilt University

We have previously investigated several endothelial properties including monolayer permeability, surface ACE activity, and the uptake of lipid soluble materials. When a mixture of functionally distinct tracers are co-injected the method produces characteristic differences in the tracer elution profiles. These patterns reflect differences in the tracer-endothelial interactions. The value of this approach is that it allows detailed study of a single cellular component which is impossible in the more complex in vivo system. The indicator-dilution experimental design has also been used in vivo as the only available means to obtain data of endothelial function from inaccessible in vivo systems. We have begun a direct comparison of elution profiles of similar materials injected into these two systems. Several similarities and differences are apparent from these two types of data. Some of these differences can be attributed to geometric dissimilarities between the two systems, however, to better compare the results of the pure endothelial system with the in vivo results, we have begun to exploit methods which can ac-

count for differences in flow and geometry. Remaining differences in elution profiles may reflect differences in endothelial function between the in vitro and in vivo systems. (NIH 40554, Whitaker Foundation, Juvenile Diabetes Foundation.)

91-44. Endothelial Uptake of Drugs, Hormones and Metabolites

J.H. Linehan and C.A. Dawson, Biomedical Engineering Department, Marquette University; Research Service, VA Medical Center and Physiology Department, Medical College of Wisconsin

Three different first-pass organ extraction approaches have been used to quantify nonlinear pulmonary endothelial uptake and/or metabolism processes. In each, the saturable process is modeled using the Michaelis-Menten equation. Direct comparison of kinetic parameter estimates from (1) serial constant substrate infusions (2) multiple indicator dilution injections of tracer substrates during the constant infusions and (3) serial multiple indicator dilution injections containing successively larger amounts of substrate delineate the impact of specific assumptions underlying the mathematical models used in each approach. The substrate used was benzoyl-Phe-Ala-Pro (BPAP). The transform of BPAP is hydrolyzed by angiotensin-converting enzyme located on the luminal surface of the pulmonary endothelium. Estimates of Vmax (maximum rate of hydrolysis) and Km (concentration at half Vmax) obtained from the three methods were not statistically different. Thus, for surface enzyme substrates the differences between approaches are not quantitatively important relative to factors contributing random errors to each approach. The choice of approach can, therefore, be made on practical grounds. (NIH HL-24349; Dept Vet. Aff.)

91-45. Effects of Capillary Recruitment and Flow Heterogeneity on Coronary Microvascular Transport in Miniature Swine

K.A. Overholser and M.H. Laughlin. Vanderbilt University and University of Missouri

We have shown earlier (J. Appl. Physiol. 67:1140, 1989) that exercise training leads to increased blood flow capacity and permeability-surface area product (PS) in the coronary circulation of the pig. However, it has not been clear to what extent flow heterogeneity and incomplete recruitment of surface area influences PS. We have now (J. Appl. Physiol., in press) extended the theory of heterogenous transport to allow for partial recruitment of functioning surface area in portions of the myocardium. In the present work, the variable-recruitment theory was applied to an exercise study. Five miniature swine were trained on a treadmill (ET) for 12 weeks; six pigs (C) were cage confined. The left anterior descending coronary artery (LAD) was then cannulated and pump-perfused under anesthesia. We used the multiple-tracer method to measure PS for EDTA and injected 15μ microspheres to mark the distribution of plasma flow F. Application of the variable-recruitment model showed that the relationship between PS and F was the same for ET and C. However, exercise training lowers hemodynamic resistance. At a given LAD pressure, LAD flow is higher and PS is larger in ET pigs compared to controls. (Supported by HL 36531.)

91-46. Estimation of Transport and Metabolic Parameters for Low Density Lipoprotein in the Rabbit Arterial Wall, In Vivo

E.D. Morris, G.M. Saidel, and G.M. Chisolm, Case Western Reserve University and The Cleveland Clinic Foundation

This work was designed to measure the contribution of the Low Density Lipoprotein (LDL)-receptor to the metabolism of LDL by the cells of the normal rabbit aortic wall. Defects in the LDL-receptor correlate with development of atherosclerosis in animals and humans. A role for receptor-mediated uptake and degradation of LDL at specific sites where atherosclerotic plaques appear, has not been established.

An experiment design was established in our earlier optimal design studies. It involved simultaneous injection of ^{125}I-LDL, as a measure of instantaneous tracer concentration, and ^{131}I-(tyramine cellobiose)-LDL, a cumulative measure of degradation. Alternatively, both tracers were also methylated to block receptor recognition of LDL. The 1-D tracer profiles were modeled by a distributed, diffusive model containing LDL-degradation terms. We found no

significant role played by the LDL-receptor in the uptake and degradation of plasma-supplied LDL by the cells of the normal rabbit aorta. This conclusion was based on fitting our model to experimental distributions obtained from serial slicing of aortic tissue radially every 10 microns. A reduced model, absent any receptor degradation terms, was adequate to describe experimental data from non-methylated and methylated tracer-pairs. This finding is contrary to work by others (Carew et al., 1984) who used a compartmental approach.

Based on our model, we are able to predict the percentage of plasma LDL destined for degradation by the inner intimal layer of the aorta and the steady state ratio of LDL degradation occurring in the intimal vs medial layers of the vessel wall, quantities of interest for researchers in atherosclerosis.

91-47. Pharmacokinetics of Acetylcholine in the Sinus Node and the Regulation of Heart Rate

F. Dexter, University of Iowa Hospitals

ACh pharmacokinetics strongly modulates vagal control of heart rate. We and others have shown, by measuring the effect of ACh on pacemaker cells, that the pharmacokinetics of ACh in neuroeffector junctions of the sinus node (NEJ) appears to be first-order. We examine mathematically the basis for this finding by considering the effects of receptor binding, diffusion, and degradation of ACh in the sinus node. We generate criteria involving experimentally determined parameters for deciding which of these processes have a significant physiological effect. We show that both the nonlinearity of ACh binding to muscarinic receptors as well as the heterogeneity of acetylcholinesterase subtypes throughout the NEJ have negligible effects on ACh pharmacokinetics. As a consequence of these and other results, the mean [ACh] at the pacemaker cells, but NOT at other sites in the NEJ, follows kinetics identical to those that would be obtained if ACh were to follow first-order linear kinetics throughout the NEJ. Heart rate can, under many circumstances, be predicted from the mean [ACh] at the pacemaker cells. Therefore, by measuring the effect of ACh on pacemaker cells, the pharmacokinetics of ACh in NEJ appears to be first-order, even though the actual kinetics are far more complicated.

II.6. *Blood Volume Control and Capacitance Control*

Organizer: Artin A. Shoukas, The Johns Hopkins University

91-48. Mean Vascular Filling Pressure – Its Meaning, Measure and Mismanagement

Carl F. Rothe and Roberto Maass-Moreno, Department of Physiology and Biophysics, Indiana University School of Medicine

The following will be reviewed:

- What does the mean vascular filling pressure purport to mean?
- What is the utility of the measure in terms of cardiovascular homeostasis?
- What techniques are used to measure it?
  - What are the assumptions – explicit and implied?
  - What are sources and possible magnitudes of error?
- What are some alternative approaches for the measure of vascular capacitance?
- Caveats.

91-49. Microvascular Volume Shift in Blood Volume Control

J.S. Lee, A. LaForte, L.P. Lee, G. Rich, and T.C. Skalak, Department of Biomedical, Engineering, University of Virginia

The microcirculation contains about 50% of the total blood volume. When the circulation experiences hemorrhage, a volume of blood can be shifted from the microcirculation to the macrocirculation. Recent experiments indicate that a decrease in the pulmonary capillary blood volume leads to a reduction in the hematocrit and hence the density of blood out-flowing from the lung. This reduction results from the Fahraeus effect in the capillary blood flow. Since the

Fahraeus effect also occurs in systemic microcirculation, we expect a reduction in the microvascular volume can lead to a reduction in the blood density but not the plasma density. When a fluid restitution develops with hemorrhage, the lower density fluid effects a reduction in plasma density. Accordingly, we could measure the change in plasma and blood density for assessing the fluid restitution and microvascular volume shift. For moderate hemorrhage (10% of blood volume) of the anesthetized rabbit, the measurement of density changes indicate that the blood volume shift composed 49% of the hemorrhaged volume while the fluid restitution 14%. These two compensations significantly minimize the effect of hemorrhage on the filing of the venous system, indicating that the shift and restitution play an important role in maintaining the cardiac function. (Supported in part by HL 40893.)

91-50. Total Vascular Compliance and Capacitance in Pacing-Induced Heart Failure

R.I. Ogilvie, D. Zborowska-Sluis, The Toronto Hospital

The dog model of chronic congestive failure induced by rapid right ventricular pacing (RVP) at 250 bpm has been widely used to study cardiopulmonary hemodynamics and tissue perfusion yet alternations in vascular compliance and capacitance have not been documented. We studied 8 pentobarbital anesthetized dogs before, during and after chronic RVP, measuring cardiac function with a pulmonary artery floatation catheter and thermodilution techniques, vascular compliance from mean circulatory filling pressures (MCFP) at 3 blood volumes during acetylcholine-induced cardiac arrest, vascular capacitance from the volume intercept at zero MCFP and blood volume by a dye technique. Chronic RVP (2–6 weeks) reduced Psa (91 to 79 mmHg) and CO (191 to 98 ml/kg/min) while increasing Pra (1.1 to 7.8 mmHg), Pw (2.0 to 16.6 mmHg) and MCFP (5.7 to 11.0 mmHg). Progressive reductions were noted in vascular capacitance (80 to 39 ml/kg), and blood volume (93 to 63 ml/kg) with a gradual return toward baseline values for capacitance and blood volume over two weeks after stopping RVP. Total vascular compliance was unaltered during the development of failure but within days of stopping RVP, it was increased above baseline values (3.2 vs 2.25 ml/kg/mmHg). Cardiac dysfunction induced by chronic RVP is associated with marked changes in total vascular capacitance and circulating volume.

91-51. The Importance of Vascular Volume Changes in Hypertension

Andrew S. Greene, Department of Physiology, The Medical College of Wisconsin

The role of changes in vascular volume was assessed in several animal models of hypertension by techniques of chronic monitoring of hemodynamic variables and servo control of body fluid volumes. Studies were conducted in both Dahl Salt-Sensitive (DSS) and Dahl Salt-Resistant (DSR) rats as well as Sprague Dawley Rats which had been subjected to a 2-stage reduction in renal mass (RRM) or a sham operation (SOC). Rats were chronically catheterized under sterile conditions and placed in a servo control scale which monitored and controlled body weight by varying intravenously delivered fluid intake. Increased sodium intake from <1 mEq/day to 8 mEq/day caused a rapid rise in arterial blood pressure (27 ± 3 mmHg) and cardiac output (94 ± 8 ml·min^{-1}·kg^{-1}, thermodilution) and blood volume (7.6 ± 0.6 ml, ^{51}Cr Red Blood Cells) in both DSS and RRM rats. DSR and SOC rats increased cardiac output and blood volume but did not become hypertensive. Servo control of body weight prevented the rise in blood pressure and blood volume in Dahl rats and greatly attenuated the response in RRM rats. We conclude that increases in blood volume associated with renal retention of sodium and water are essential in the development of hypertension in DSS and RRM rats.

91-52. Adrenergic and Cholinergic Regulation of Splanchnic Intravascular Volume and Cardiac Output

David L. Rutlen, Yale University School of Medicine

Whether selective autonomic receptor stimulation influences cardiac output (CO) via changes in splanchnic intravascular volume (SIV) was assessed using radionuclide imaging. In anesthe-

tized dogs, phenylephrine (.04–.08 mg min^{-1} iv) for 20 min was associated with a 12 ± 2% ($p < 0.001$) increase in CO and a decrease in total SIV of 431 ± 95 ml ($p < 0.001$) due entirely to decreases in splenic and extrahepatosplenic volume of 26 ± 8% ($p < 0.001$) and 7 ± 2% ($p < 0.001$). In eviscerated animals, CO decreased 30 ± 2% ($p < 0.001$) with phenylephrine. With isoproterenol (.006 mg min^{-1} iv) total SIV decreased 12 ± 1% ($p < 0.001$, 5.2 ± 1.6 ml kg^{-1}) due entirely to a decrease in splenic intravascular volume of 24 ± 3% ($p < 0.001$). The isoproterenol associated SIV decrement was dependent upon beta-2 and alpha adrenergic receptor stimulation but not beta-1 adrenergic stimulation. If beta-1 adrenergic stimulation was sufficiently minimized with metoprolol, splenectomy attenuated the isoproterenol associated CO increment. With acetylcholine (.005 mg kg^{-1} min^{-1} iv) total SIV decreased 4.9 ± 1.0% ($p < 0.001$, 65 ± 14 ml) due entirely to a decrease in splenic volume of 10.3 ± 2.0% ($p < 0.001$), and CO increased from 1916 ± 190 to 2290 ± 230 ml min^{-1} ($p < 0.001$). Splenectomy or evisceration did not alter the acetylcholine associated CO response. Thus, alpha and beta adrenergic and cholinergic stimulation act to decrease total SIV. Only the SIV decrements associated with alpha or beta adrenergic stimulation, under conditions of minimal beta-1 adrenergic stimulation, act to enhance CO.

91-53. Carotid Baroreflex Control of Hormonal Release in Conscious and Anesthetized Dogs

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The carotid baroreflex represents a major control system involved in the restoration of blood pressure following external disturbances to the cardiovascular system. The influence of this system on hormonal release has been extensively studied in anesthetized preparations, but has not previously been studied using an open loop experimental approach in conscious animals. Carotid baroreceptors are isolated and perfused at controlled carotid sinus pressures (CSP) in the conscious dog. Open loop experiments have been conducted in both anesthetized as well as conscious dogs in order to assess the role of the carotid baroreflex in the release of various hormones. In anesthetized, vagotomized dogs, decreases in isolated carotid sinus pressure (CSP) caused reflex induced increases in plasma levels of β-endorphin and arginine vasopressin (AVP). A similar increase in AVP is seen at low levels of CSP in both acutely prepared anesthetized, as well as in chronically prepared conscious dogs. Increases in peripheral cortisol in response to decreases in CSP have been demonstrated in the conscious dog. These results imply an important role for the carotid baroreflex in the control of hormonal release. (Supported by NIH Grant HL-38316.)

91-54. Reflex Control of Liver Capacitance after Hemorrhage

C.V. Greenway, Department of Pharmacology, University of Manitoba

Cats anesthetized with pentobarbital were subjected to slow hemorrhage (1 ml min^{-1} kg body $weight^{-1}$) until arterial pressure fell to 60 mm Hg. After a 6 min wait, the blood was reinfused. This procedure was repeated 3 times in each cat. In intact cats, a hemorrhage of 21 ± 2 ml/kg was required. After splanchnic nerve section, this volume was reduced to 8 ± 1 ml/kg ($p < .001$). Active contraction of hepatic capacitance vessels was demonstrated in the intact cats by portal pressure/hepatic volume plots. Hepatic blood volume was reduced by 14 ± 3 ml/100g liver at the same portal pressure after hemorrhage compared to before. This was reduced after splanchnic nerve section to 6 ± 1 ml/100g liver ($p < .02$). Previous work and this study suggest that this active contraction did not involve arterial baroreceptors, vagal or cardiac afferent nerves, but required a decrease in pressure in the hepatic veins or neighbouring inferior vena cava. We postulate a reflex control of the hepatic capacitance vessels mediated through the splanchnic nerves from pressure-receptors in the hepatic veins. Previous findings of no response to hemorrhage and a high resting sympathetic tone (1987 Can. J. Physiol. Pharmacol. 65:2168) were due to maintenance of hepatic venous pressure at zero by an extracorporeal long-circuit. This reflex did not control splenic contraction after hemorrhage. (Supported by Manitoba Heart Foundation.)

91-55. Mechanisms Involved in the Restitution of Blood Volume and Plasma Protein after Hemorrhage as Assessed Experimentally and with a Mathematical Model

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The early restitution of blood volume after hemorrhage involves the movement of protein-free fluid into the vascular compartment. Further restitution of volume and of plasma protein requires the release of several hormones after hemorrhage. Although the input from cardiovascular afferents during hemorrhage appears primary in eliciting these hormonal responses, previous stimulus history and behavioral factors modulate the responses and the associated late restitution of plasma protein. Previously, we proposed that the hormonal responses to hemorrhage elicit an increase in the amount of solute in the interstitium that then acts osmotically to expand this compartment with fluid from the intracellular space. A model suggested that this expansion increased interstitial pressure to drive the restitution of plasma protein. During shock after large hemorrhage, this process may be reversed by the movement of sodium into cells possibly because of inhibition of the Na-K ATPase. Accordingly, we have expanded our mathematical model to include the intracellular compartment and the dynamics of Na and K. This new model suggests that, in addition to an increase in nonionic extracellular solute after hemorrhage, a bound pool of monovalent cations in the interstitium releases free cations to buffer their extracellular concentrations and to enhance the expansion of the interstitium. Prolonged inhibition of the Na-K ATPase in the model impairs the restitution of plasma protein after large hemorrhage, as has been observed experimentally. (Supported in part by NIH grant GM-27946.)

91-56. Importance of Reflexly Induced Changes in Regional Capacitance in the Control of Cardiac Output and Blood Pressure

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Reflexes which are responsible for the safeguard of blood pressure and cardiac output during hemorrhage must operate on the arterial as well as on the venous side of the circulation. While arterial vasoconstriction can limit blood loss and partially restore blood pressure, the volume loss can be replaced through sympathetic changes in regional vascular volumes, which will also help maintain cardiac output and blood pressure. These changes in regional volumes can be effected through a decrease in unstressed volume (V_o), in venous compliance (C_v), in venous resistance (R_v), or by an increase in fractional flow to fast time constant beds. In dogs on circulatory bypass at constant cardiac output, regional blood volumes were measured and pressure-volume curves were constructed. Studies on the reflex response to decreases in carotid sinus pressure revealed that volume is recruited from the splanchnic region only. These volume changes are the result of a reduction in V_o, and a large decrease in R_v. Increases in fractional flow to the splanchnic bed and in its C_v were more than compensated for by the change in R_v. Hexamethonium totally abolished the changes in unstressed volume, but only half of it could be reversed by α-adrenergic receptor blockade. Studies on the reflex response to increases in core temperature revealed that V_o of the splanchnic bed is reduced during heat stress with no change in R_v, C_v or in distribution of blood flow. Hexamethonium abolished these changes in V_o, but both α- and β-adrenergic blockade were ineffective. A possible mediator for these changes in V_o is neuropeptide Y (NPY) for infusions of NPY decreased splanchnic V_o without affecting C_v, R_v or the distribution of blood flow. In conclusion, changes in venous resistance play an important role in the control of splanchnic volume during hypotension, and NPY may play an important role in the control of splanchnic V_o.

91-57. Arginine Vasopressin Increases Whole Body Capacitance in Anesthetized Cats

D.S. Martin and J.R. McNeill, Departments of Pharmacology, University of Texas Health Science Center and University of Saskatchewan

Arginine vasopressin (AVP) is potent vasoconstrictor agent in vitro, yet is an unremarkable pressor agent when administered to animals with intact autonomic function. The pressor

effects of AVP appear to be buffered by decreases in cardiac output. Since vascular capacitance plays a major role in the control of cardiac output, the present study was undertaken to determine if AVP elicits changes in vascular capacity. Experiments were carried out using the constant-flow reservoir technique in anesthetized cats. AVP was infused (1–100 ng/kg/min) intravenously before and after impairment of autonomic function with a ganglion blocking agent, pentolinium tartrate (2.5 mg/kg + 0.25 mg/kg/min). During the AVP infusions plasma AVP concentrations ranged from 70 to 4000 fmol/ml. There was no difference in the dose-plasma concentration relationship between intact and ganglion blocked cats. In cats with intact autonomic function, the AVP infusions were associated with dose-dependent decreases in reservoir volume (approximately 1.6–7.8 ml/kg) reflecting increases in whole body capacity. Systemic compliance was not changed during the AVP infusions. Pretreatment with the ganglion blocking agent attenuated the AVP induced decreases in reservoir volume by approximately 90%. These results suggest that elevations in the circulating concentrations of AVP elicit reflexly mediated increases in whole body capacity. These increases in capacity do not involve an increase in systemic compliance. Thus, the decreases in cardiac output associated with the administration of AVP may be mediated, at least in part, via reflex increases in unstressed vascular volume. (Supported by MRC Canada. D.M. supported by a Fellowship from MRC Canada.)

91-59. The Effects of Phentolamine and Propranolol on The Changes in Vascular Capacitance and Resistance Caused by The Carotid Sinus Baroreflex

K. Shigemi, M.J. Brunner, and A.A. Shoukas, The Johns Hopkins Medical School, *University of Maryland School of Medicine*

We examined the contribution of α and β adrenergic receptor mechanisms to the changes in systemic vascular capacitance and resistance caused by the carotid sinus baroreflex in anesthetized vagotomized dogs. The carotid sinuses were isolated from the systemic circulation and perfused with controlled pressures. A constant flow cardiopulmonary bypass preparation was used in which the change in external reservoir volume was continuously measured. The change in vascular capacitance was determined from the changes in arterial compliance, venous compliance, and the reservoir volume when carotid sinus pressure was reduced from 200 to 50 mmHg without any receptor antagonist, with either α(phentolamine) or β(propranolol) antagonist, and with both α and β antagonists. The change in vascular capacitance was 25 $\pm$ 10 ml/kg with no antagonist. The capacitance change was reduced by 74% with phentolamine, by 33% with propranolol, and by 75% with both antagonists. Vascular resistance was increased by 0.60 $\pm$ 0.21 (from 0.72 $\pm$ 0.16 to 1.34 $\pm$ 0.27) mmHg·min·kg/ml when carotid sinus pressure was decreased under control condition. The resistance change was reduced by 80% with phentolamine, increased by 20% with propranolol, and reduced by 60% with both antagonists. These results suggest that capacitance and resistance vessels are predominantly activated by α receptors. (Supported by NIH Grant #HL19039 and #HL14529.)

II.7. *Bioengineering Aspects of Microvascular Flow in Ischemia and Shock*

Organizer: Geert W. Schmid-Schönbein, University of California, San Diego

91-60. Adherence Strength of T Cells to Planar Membranes Containing LFA-3 Molecules

A. Tözeren, K.-L.P. Sung, L.A. Sung, M.L. Dustin, P.Y. Chen, T.A. Springer and S. Chien, Biomedical Engineering Program, Catholic University of America, Harvard University, and University of California, San Diego

Cell adhesion plays a fundamental role in cell motility and immune response. We investigated the adhesion between a Jurkat cell, chosen for its high expression of CD2, and a glass-supported planar membrane containing either the laterally mobile, lipid-anchored isoform (GPI LFA-3) or the immobile, transmembrane isoform (TM LFA-3) of the counter-receptor LFA-3 at the same concentration of 2,000 LFA-3 molecules/μm^2 by using a novel micromanipula-

tion method. In this technique, the pipette holding the cell is micromanipulated in the direction perpendicular to a glass-supported lipid bilayer reconstituted with a given type of surface adhesion molecules. In experiments using the planar membrane containing GPI LFA-3, the adhered Jurkat cell deformed extensively in response to the pipette force, as the cell detachment proceeded by peeling at the edges of the contact area. When the cell elongation in the direction of the pipette reached a maximum, the cell separated rapidly from the planar membrane. In experiments using the planar membrane containing TM LFA-3, Jurkat cells detached with little resistance to micromanipulation and without changing their round shape. Our experimental data showed that the aspiration pressure required to detach a Jurkat cell from a membrane containing mobile LFA-3 is about one order of magnitude greater than that for the immobile LFA-3.

91-61. Deformation and Flow of Neutrophils in Micropipets

R.M. Hochmuth, D. Needham, H.P. Ting-Beall, and D. Zhelev, Department of Mechanical Engineering and Materials Science, Duke University

The undeformed human neutrophil in the resting, passive state is shaped like a sphere with a diameter of about 8 μm. Thus, it must deform significantly in order to flow through the small capillaries (~4 μm) of the body. To model and understand this process we study the deformation and flow of neutrophils into uniform and tapered glass pipets with pipet openings on the order of 4 μm. We find, as others have, that the passive neutrophil does not behave as a "standard solid" but instead deforms and flows smoothly like a liquid drop. This liquid drop has a persistent "surface" tension that is very small—about 0.02–0.04 dyn/cm. In some instances the tension appears to increase as the surface area of the cell is expanded. The apparent viscosity of this liquid drop is very large—about 10^3 poise—and appears to decrease somewhat at higher rates of flow. For slow flows over long periods of time the cell can "activate" without sticking to the pipet wall and without forming pseudopods. In these instances the cell behaves as a viscoelastic gel with a much larger resistance to deformation and flow than that exhibited by a resting cell. (Supported by NIH-NHLBI Grant HL 23728.)

91-62. The Effect of Cytoskeletal Structures on the Morphology of T Cells Adhering to a Planar Substrate

L.H. Mackie, K. Thornton, and A. Tozeren, Biomedical Engineering Program, Catholic University of America

In this study, we used a novel video-microscopy technique to test the validity of the cortical shell–liquid core model of Yeung and Evans (Biophysical J. 56:139–150, 1989) for the case of white blood cells adhering to a planar substrate. In this method, the cells were allowed to settle on a glass-chip that was positioned perpendicularly on the bottom of a cell chamber. The chamber was then placed on the stage of an inverted microscope, and the side views of the cells adhering to the glass chip were videotaped. The adhesion of T lymphocytes, maximally stimulated with phorbol 12-myristate-13 acetate (PMA), to a glass supported lipid bi-layer containing 2000 ICAM-1 molecules/μm^2 was considered first. The videotapes of the time course of the T cell interaction with the planar membrane indicated that these cells formed the shape of a liquid drop (part of a sphere) as predicted by the model. The addition of Cytochalasin B (CB), a drug which disrupts the actin filaments, did not induce cell detachment. Next, we considered the nonspecific adhesion of HL-60 promyelocytic neutrophils to clean glass. After the addition of CB, the cell shapes deviated significantly from the corresponding model predictions. The results suggest that the cell cortex as well as other cytoskeletal structures determine the cell morphology.

91-63. Capillary Endothelial Cell Swelling in Low-Flow Ischemia

M.C. Mazzoni, M. Intaglietta, E.J. Cragoe, Jr., and K.-E. Arfors, Pharmacia Experimental Medicine

Acute episodes of low-flow ischemia, including hemorrhagic shock, vascular occlusion, and local tissue trauma, are frequently encountered in clinical medicine. Intravital microscopy stud-

ies in the rabbit tenuissimus muscle have previously demonstrated capillary narrowing on the order of 25% during hemorrhagic shock owing to swollen endothelium. The swelling is attributed to an influx of Na^+ and water, and may be caused by the diminished capillary perfusion, the induced metabolic acidosis, or a combination of the two. In shock experiments (40% single-withdrawal hemorrhage, one hour duration), inhibition of the Na^+/H^+ exchanger but not the Na^+ channels by pretreatment with specific analogs of the drug amiloride, was found to prevent endothelial cell swelling. To study the flow factor, experiments were done in which muscle blood flow was reduced by proximal arterial occlusion for one hour to shock levels as measured by laser Doppler flowmetry, with systemic blood pH maintained yet local tissue pH becoming acidic. There was no apparent cell swelling during occlusion, but on its release a slight narrowing (<5%) was observed, along with a modest decrease in systemic pH presumably from the washout of hindlimb tissue metabolites. These results suggest that an acidic endothelial cell milieu and not low-flow *per se* induce endothelial cell swelling via pH regulation. Narrowed capillaries with elevated resistances may hinder reflow efforts. Infusion of isotonic fluids or correction of blood acidosis with bicarbonate after shock, both conventional treatment modalities, do not affect the narrowing. To date, only hypertonic saline reinfusion has been found to rectify diameter and reinstate flow in shock-narrowed capillaries.

91-64. In Vivo Measurements of Leukocyte Rheology and Capillary Plugging

Kevin C. Warnke and Thomas C. Skalak, Department of Biomedical Engineering, University of Virginia

Quantitative measurements of leukocyte capillary plugging in vivo in rat spinotrapezius muscles were used to determine the effects of leukocytes on blood flow in the microcirculation and to estimate effective WBC viscosity. The frequency and duration of leukocyte plugs were observed in all capillary branches in a group of 23 terminal arteriolar trees. The average number of plugs, normalized to account for the size of the tree, was applied to a previously developed model relating capillary plugging by leukocytes to resultant network resistance increases and yielded an average increase in resistance of 3.1 percent due to the WBCs. The distribution of resistance increases was log-normal, with 78% of the trees exhibiting a resistance increase of less than 3%. All of the resistance increases greater than 3% were due to apparently adherent leukocytes that plugged individual capillaries for over 30 seconds. Measurements of the durations of 390 WBC plugs, cell and capillary dimensions, and the arteriolar pressures at the plugging sites were applied to a biomechanical model of leukocyte plugging, yielding a log-normal distribution of effective WBC viscosity with a median value of 228 Poise. 3.9% of the WBCs had calculated viscosities greater than 3000 Poise, indicating activation of this small fraction of cells. The median duration for the 1686 measured plugs was 0.1 seconds. (Supported by NIH Grant HL-39680 and NSF Graduate Fellowship)

91-65. Leukocyte Deformability and Dynamics in the Microcirculation in the Low Flow State

H.H. Lipowsky, Bioengineering Program, Penn State University

The functional behavior of the leukocyte (white blood cell, WBC) in the microcirculation is often accompanied by undersirable effects on microvascular blood flow which arise from capillary plugging by the less deformable WBCs (compared to red blood cells, RBCs) and their preferential adhesion to the endothelium (EC) of postcapillary venules, which is an essential step in the inflammatory process. Both of these processes may adversely affect microvascular flow as pressure gradients are diminished in the low flow state, although the details of whether plugging or adhesion has a greater effect remains to be determined. To address this question, measurements of the transit time of fluorescently labelled WBCs and RBCs throughout successive microvascular divisions (obtained by indicator dilution techniques under fluorescence microscopy of cremaster muscle) were performed and suggest that the process of cell entrapment within the capillary orifice may proceed to a greater extent without compromising the total throughput of the microvascular network. As capillaries become plugged, alternate pathways for flow are recruited to satisfy tissue metabolic demands. Stimulating WBC-EC adhesion in the normal flow state suggests that microvascular flow is adversely affected by WBC-EC adhesion to a greater extent than capillary plugging by rigid WBCs. Adhesion of as few as 12

WBCs per 100 μm along the length of a 40 μm diameter venule is sufficient to raise flow resistance two-fold. The adverse effects of both capillary plugging and venular obstruction may be exacerbated by reductions in WBC deformability attendant to WBC activation during inflammation. Thus, the relative roles of capillary plugging and WBC-EC adhesion need to be delineated in terms of both flow reductions and the magnitude of the inflammatory process. (Supported by USPHS NHLBI Research Grants HL-28381 and HL-39286.)

91-66. Leukocyte Involvement in Cerebral Ischemia and Reperfusion Injury

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The purpose of this study was to evaluate the contribution of leukocytes to cerebral ischemic damage. Comparisons were made of cortical electrical activity, somatosensory evoked potentials and infarct size (measured by triphenyl tetrazolium chloride [TTC] staining) in rats with normal leukocyte counts and rats rendered leukopenic by vinblastine injection. There was complete cessation of EEG activity, and the cortical peak of the SSEP was lost during the ischemic period in the control animals. In the leukopenic animals there was maintenance of EEG activity and the cortical peak of the SSEP was preserved during the ischemic period. Postischemic SSEPs were also improved in the leukopenic animals. Infarct size was measured one hour and fifteen minutes after a one hour period of ischemia. The percent of the brain surface area infarcted in the control animals was 71% compared to 21% in the leukopenic animals. Therefore both electrical and morphologic evaluation indicated better preservation of the leukopenic animals.

91-67. Microvascular Changes after Cardioplegia Solution Infusion and Blood Reperfusion: Effects of Adenosine

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The hypothesis that hyperkalemic, crystalloid cardioplegia solutions damage the microvasculature, leading to decreased microvascular flow and increased neutrophil accumulation during reperfusion, was tested in a model system. Intravital microscopic observations were performed during a 20 minute perfusion of the hamster cremaster with cardioplegia solutions (10°C) via the femoral artery with the iliac occluded, and during a subsequent 2 hour blood reperfusion period (iliac open). Arteriolar vasoconstriction (27% decrease in diameter, $p < 0.05$) and a 25% decrease in the density of perfused capillaries ($p < 0.05$) occurred during reperfusion in animals receiving crystalloid cardioplegia (16 mEq K^+) compared to control animals (no cardioplegia solution given). Rolling neutrophils accumulated on venular endothelium in cardioplegia treated animals (250% increase, $p < 0.05$) and extravascularly (myeloperoxidase levels 2.0 ± 0.5 U/g vs. 1.3 ± 0.3 U/g in control, $p < 0.05$). The addition of adenosine (10^{-4} M) and albumin (2% by volume) to the cardioplegia perfusate, accompanied by the administration of adenosine (10^{-4} M) during reperfusion, produced arteriolar vasodilation (34% diameter increase, $p < 0.05$) and inhibited extravascular neutrophil accumulation (myeloperoxidase level of 1.5 ± 0.2 U/g, $p < 0.05$ vs control), but capillary perfusion was still significantly diminished (28% decrease, $p < 0.05$). We conclude that microvascular blood flow decreases and neutrophils accumulate in tissues during blood reperfusion following infusion of hyperkalemic crystalloid cardioplegia solutions. Adenosine partially reversed these changes by dilating arterioles and decreasing neutrophil accumulation. The microvascular changes seen were independent of perfusate temperature, and were most likely due to endothelial dysfunction induced by the high potassium content and crystalloid composition of the cardioplegia solution.

91-68. The Role of Neutrophils in Tissue Injury During Hemorrhagic Shock

J. Barroso-Aranda and G.W. Schmid-Schönbein, Department of AMES-Bioengineering, University of California, San Diego

Polymorphonuclear neutrophils (PMNs) are large and stiff circulating cells, they can adhere readily to the vascular endothelium, and when activated elaborate oxygen free radicals and pro-

teolytic enzymes. Recent evidence shows that PMNs play an important role in capillary stasis and tissue injury. Capillary plugging by PMNS has been shown to be the underlying mechanism for the no-reflow phenomenon in hemorrhagic shock. When rats were depleted of PMNs by intraperitonial injection of a polyclonal antibody directed against PMNs, the animals survived 100% following even severe shock protocols. Exposure to a milder form of hemorrhagic shock causes untreated animals to survive in part, so that in spite of identical treatment a survivor and a non-survivor group can be identified. In an attempt to distinguish between these two groups of animals, the state of spontaneous activation of circulating PMNs before hemorrhagic shock was measured with the nitroblue tetrazolium (NBT) test, a measure for the number of PMNs producing superoxide radicals. For rats which initially had high circulating PMN activation the experimental procedure was lethal. Animals which before hemorrhage had low circulating PMN activation but during the course of hypotension significantly elevated their circulating count, have low probability for survival. Survivors had low circulating PMN activation before bleeding and during the hypotensive period. If the rats were pretreated with pentoxifylline, which acts on PMNs to decrease adherence to the endothelium, to suppress oxygen radical production, and to decrease degranulation, such animals showed significantly improved survival following hemorrhagic shock. These results suggest that PMNs play an important role in the pathophysiology of hemorrhagic shock. (Supported by USPHS Grant HL 10881, HL 43026, and Hoechst-Roussel Pharmaceuticals, Inc.)

II.8 *Thrombosis and the Circulation*

Organizer: Vincent T. Turitto, Memphis State University

91-69. Platelet Concentration Profiles during Reaction

I. John Khan and Eugene C. Eckstein, University of Miami

Several groups have demonstrated that blood flow is often associated with a near wall excess of platelets. Our group's recently developed simulation program was used to model the initial stages of platelet deposition on reactive smooth surfaces. The program described platelet motions as having drift and diffusive components in the lateral direction and a convective component in the axial direction. The simulations showed that the platelet concentration profile contains a near-wall peak superimposed on a concentration gradient. For diffusion-limited transport to the surface, the peak height was relatively short.

To study these events experimentally, our freeze-capture technique was modified to include classical histochemistry. The inner surface of a hollow fiber from a high-flux dialyzer (Hospal Filtral with AN69 membrane) is coated with collagen, and then blood is passed through for short periods of 1 to 2 minutes. The fiber is frozen, cryo-fixed with glyoxal (or with tannic acid), dehydrated in ethanol, and embedded in either LR White or PolyBed 812 resin. Conventional thin-section microscopy techniques are used to assay platelet numbers and involvement at the wall. Ongoing work seeks to combine methods involving serial sections and staining with monoclonal antibodies. This combination should allow us to distinguish between platelets that are attached to thrombi, those that are activated but free, and those which are inactive. We will report on the progress of these studies, and how they complement other investigator's studies of thrombus growth on reactive surfaces.

91-70. Functional Expression of Tissue Factor by Fibroblasts and Activated Endothelial Cells in an In Vitro Flow System

Eric F. Grabowski, Cornell University Medical Center

The expression of tissue factor (TF) by a variety of vascular cell types under physiologic flow conditions is critical to Factor X (FX) activation and in vivo clotting. In a parallel-plate flow chamber (volume 40 μl), therefore, we mounted monolayers of human embryonic fibroblasts (FB's) or IL-1α (5U/ml·4 hrs)—stimulated human umbilical vein endothelial cells (EC's). Inflow buffer contained 10 nM FVII, 100 nM FX, and a chromogenic substrate for the amido-

lytic assay of FXa. With FB's as a positive control, FXa production (product of outflow chromophore concentration and flow rate) increased significantly ($p < 0.001$; Table) with shear rate. With EC's, production was not detectable. In the presence of an antibody directed against the lipoprotein-associated coagulation inhibitor (anti-LACI; courtesy of Dr. G. Brosz), however, FXa production with EC's increased 40-fold over the range of shear rate studied ($p < 0.001$). A monoclonal antibody to human TF blocked 70% or more of production with either cell type. Experiments with supernatant vs washed EC's confirmed that FXa is not released into the flowing buffer. Indeed, chromogenic substrate added to the outflow instead of the inflow failed to detect any appreciable FXa. We conclude that the inability of activated EC's to generate FXa in the presence of FX and FVII is due to EC-derived LACI. With anti-LACI (in simulation of a hypercoagulable state?), FXa production is measurable, shear rate augmented, and cell associated.

Further studies will focus on other activating agents (e.g., tissue necrosis factor), assay for LACI itself, assay for TF mRNA, and incorporation of TF expression in a flow model (red cell suspension and platelets) of platelet adhesion/aggregation to locally injured endothelium.

91-71. The Relative Effects of Inhibition of Platelet Recruitment Mediated by ADP, TXA2, and Thrombin

Jeffrey A. Hubbell and William R. Wagner, Department of Chemical Engineering, University of Texas

Previous mathematical modeling of the concentrations of platelet-derived platelet-active species near a growing thrombus in heparin-anticoagulated whole blood suggested that thrombin may dominate this process, that ADP may be somewhat important, and that thromboxane A2 (TxA2) may be less important. Experimental observations in heparinized whole human blood at a wall shear rate of 1000 s^{-1} using various inhibitors of thrombin confirmed thrombin's major role, even in the presence of heparin. A large fraction of its activity was attributable to the stabilization of the thrombus by locally formed fibrin, as was observed by inhibition of this process. The instability of the thrombi to flow and the microembolization of thrombus fragments from the tops of thrombi was deduced from the shape of the axial deposition curve, from the shape of the temporal deposition curve, and by direct microscopic observation. Inhibition of ADP-mediated platelet recruitment also resulted in a large reduction of platelet deposition on collagen. Inhibition of TxA2-mediated recruitment resulted in reduction of deposition that was due more to a reduced hydrodynamic stability of the growing thrombi than to a reduced level of platelet attachment to the surface or aggregate structures.

91-72. Hydrodynamic and Mass Transport Regulation of the Fibrinolytic Enzymes

Scott L. Diamond and Jung-He Wu, Department of Chemical Engineering, State University of New York

Secretion of tissue plasminogen activator (tPA) by human umbilical vein endothelial cells is elevated by arterial levels of shear stress. Further study using a polymerase chain reaction technique has shown that the tPA mRNA level in cells exposed to arterial shear stress of 25 dynes/cm^2 is elevated compared to control cultures. We are conducting experiments to determine if transcriptional regulators in the cellular nucleus are induced by hydrodynamic forces, thus causing the observed increase in tPA mRNA.

To understand the role of elevated levels of tPA near the fluid/fibrin clot boundary, we have carried out a theoretical and experimental investigation of the heterogeneous reactions involved in fibrinolysis with emphasis on the advective/dispersive transport of fibrinolytic agents into preformed clot structures. In visualizing the diffusive movement of proteins in these clots with fluorescence microscopy, the effective diffusivity was not affected by steric hinderance, however the effect of fibrin binding greatly reduced the mobility of tPA. A simulation of multicomponent transport and reaction will be presented which demonstrates the sensitivity of the fibrinolytic reactions to the initial plasminogen concentration of the clot. Also, the role of plasmin mobility will be discussed in terms of inner-fibrin strand steric hinderances and diffusion-limited fibrinolysis.

91-73. Thrombin Generation Profiles in Blood Under Flow Conditions

Cynthia H. Gemmell, University of Toronto

A new in vitro assay system designed to attain greater understanding of the physical variables (shear rate, flow rate, and tube diameter) important in thrombin production has been developed. It is expected that the test system will also serve to compare biomaterials. The test system is an improvement over existing clotting time tests in that the effect of surface on thrombin production during flow will be assessed.

Experimentation involves the mixing of two calcium streams directly into a citrated plasma or whole blood stream prior to flow within a tube segment. Samples are collected in EDTA at the outlet with time and assayed for thrombin using a very sensitive fluorogenic substrate, BOC-val-pro-arg-7-amido-4 methyl coumarin.

The relative importance of flow rate, shear and contact time are currently under investigation for polyethylene tubes. Glass tubes (1.33 mm ID, 25 cm L) at low shear rates (70 sec^{-1}, 25 sec contact time) resulted in an exponential increase in thrombin concentration over a 15-minute period compared to very low steady state thrombin levels for polyethylene tubing.

91-74. Increased Viscosity Decreases the Activation of Factor X by Tissue Factor–Factor VIIa in a Tubular Reactor

S. Gir, Y. Nemerson, and V. Turitto, Biomedical Engineering, Memphis State University and *Department of Medicine, Mt. Sinai Medical Center*

The effect of shear stress on the ability of tissue factor–factor VIIa to activate factor X was studied in a continuous flow tubular reactor. Recent studies have shown that the steady state production of factor Xa at high substrate concentrations (V_{max}) was strongly dependent on wall shear rate (γ_w) increasing 3-fold as γ_w increased from 57 to 600 sec^{-1}. The reactor is a capillary tube (0.27 mm ID) whose inner surface was coated with a phospholipid (70:30 mole % phosphatidyl choline to serine) bilayer which contains a 1 to 50,000 mole ratio of tissue factor. Factors VIIa (10 nM) and X (100–800 nM) were perfused through the reactor for $\gamma_w = 100$ sec^{-1} for up to 20 min. Factor Xa in the effluent was determined by chromogenic assay. Shear stress at the wall was investigated at 1 and 3 dynes/cm^2, at a constant γ_w, by increasing the perfusate viscosity with sucrose (40% wt) or glycerol (30% wt). The increase in viscosity, and correspondingly the wall shear stress, produced a 3-4 fold *decrease* in the outlet concentration of factor Xa. Increasing the factor X concentration from 100 to 800 nM increased the outlet concentration by a factor of 7; however, the relative concentrations at the two viscosities were comparable. These findings are in contrast to the effect observed at increased wall shear rates and suggest one of several possibilities: (1) a physical inhibition of TF-VIIa by wall shear stress; (2) reduced diffusional transport of factor X to TF-VIIa; or (3) a direct chemical affect of sucrose or glycerol on TF-VIIa. The last point is somewhat unlikely as the activation of factor X by tissue factor-factor VIIa in a test tube was found to be unaffected by the presence of glucose or sucrose.

II.9. *Respiratory Mechanics: Models of Micro-Scale Phenomena I. Fluid Mechanics*

Organizers: Roger D. Kamm and Jeff Fredberg, MIT and Harvard University

91-76. Adsorption, Area Compression and Hysteresis of Pulmonary Surfactant Films

Samuel Schürch and Hans Bachofen, Respiratory Research Group, University of Calgary, and Department of Anatomy, University of Berne

Surface tension-area relations from pulmonary surfactant were obtained with a new apparatus that contains a leak-free captive bubble of controllable size. Rat pulmonary surfactant

was studied at phospholipid concentrations of 50, 200 and 400 μg/ml. At the highest concentration, adsorption was rapid, reaching surface tensions below 30 mN/m within 1 sec, while at the lowest concentration, approximately 3 min were required. Upon a first quasi static or dynamic compression, stable surface tension below 1 mN/m could be obtained by a film area reduction of approximately 50%. After 3–4 cycles the surface tension-area relations became stationary, and the tension fell from 25–30 to approximately 1 mN/m for a film area reduction of less than 20%. Hysteresis became negligible, provided the films were not collapsed by further area reduction at minimum surface tension. After only 3–4 consecutive cycles, surfactant films exhibited the low surface tensions, collapse rates and compressibilities characteristic of alveolar surfaces in situ or of monolayers from pure dipalmitoyl phosphatidylcholine. Surface tension and area are interrelated in the captive bubble which may promote low and stable surface tensions. If the surface tension of the captive bubble suddenly increases ("click"), the bubble shape changes from flat to more spherical. Hence there is an isovolumetric decrease in surface area which in turn decreases the surface tension. This feedback mechanism may also have a favorable effect in stabilizing the alveolar surface area of the lung.

91-77. Surfactant Spreading on Thin Liquid Films

James Grotberg, David Halpern, and Oliver Jensen, Departments of Biomedical Engineering and Anesthesia, Northwestern University

The dynamics of surfactant spreading in either droplet or frontal configurations is approached theoretically for thin layer substrates such as the lung's liquid lining. The intent is to understand the fundamental fluid and interfacial mechanics which determine spreading rates, flow patterns, transport behavior of soluble contaminants and criteria for film rupture. Potential applications are generally in the area of inhalation drug therapy and, at the present stage of development, particularly in surfactant replacement treatment for neonatal respiratory distress syndrome by either intra-airway bolus or aerosol modalities. Results of these models indicate that spreading due to surface tension gradients leads to an outward traveling wave disturbance of the air-liquid interface. The induced flows within the film may be bi-directional, and under specific circumstances the wave trough is close enough to the lower boundary that a film rupture instability occurs which halts the spreading. For soluble surfactants which may be absorbed into the lower wall, a droplet first will spread but then will retract due to a reversal of surface tension gradients. When the surface Peclet number is sufficiently large, a shock develops at the leading edge of the spreading surfactant, and the shock speed and structure dominate the system. (Supported by NIH grants HL-01818 and HL-41126.)

91-78. The Effects on Airway Closure of Pulmonary Surfactant and Changes in Lung Volume

R.D. Kamm, D. Otis, and M. Johnson, Fluid Mechanics Laboratory, Department of Mechanical Engineering, Massachusetts Institute of Technology

Numerical simulations have been conducted to study airway closure in the small pulmonary airways due to a liquid film instability creating an obstructing meniscus, taking into account the effects of changing lung volume and the presence of pulmonary surfactant. The model consists of a tube of finite length and uniform circular cross-section, lined with a thin liquid layer. Our initial calculations simulating a linear fall in lung volume with time but in the absence of surfactant, indicated that closure would occur as soon as the conditions existed for the initiation of the fluid dynamic instability. This condition was reached at a lung volume of about 26% TLC when the liquid film thickness was 10 μm at TLC. Rates of expiration within the normal physiologic range had no significant effect on closing volume. Including an insoluble surfactant, however, changed the results significantly. Depending on the minimal surface tension reached by the liquid film in the airways during expiration, we found that airway closure could be significantly delayed. For example, with a surface tension of 20 dynes/cm at Total Lung Capacity (TLC), and falling to a minimum value of 2 dynes/cm (and using an empirically-derived

relationship between surfactant concentration and surface tension for pulmonary surfactant) closure could be delayed to significantly lower lung volumes, as low as about 14% TLC with very rapid lung deflations. These results also raise the prospect of closure on *inspiration*, rather than expiration, due to the abrupt increase in surface tension as the film begins to expand. (This work supported by a grant from the NHLBI HL33009.)

91-79. Kinetics of Aerosol Deposition in the Pulmonary Aginus

Akira Tsuda and Jeffrey J. Fredberg, The Biomechanics Institute and Harvard School of Public Health

We tested the hypothesis that the complex structure of the alveolar duct has a significant influence upon the process of aerosol deposition. A geometric model of the alveolated duct was generated and the velocity field of carrier gas in that conduit was solved numerically. A Monte Carlo analysis of aerosol kinetics was used. The dynamic behavior of aerosol particles was described by the Langevin equation (Newton's second law of motion including random Brownian force) and their trajectories were computed by successively integrating the Langevin equation. Conditional probabilities of deposition site for a given initial condition were computed. Based on these computations, the deposition process in alveolated ducts was studied over physiologically relevant conditions. The analysis indicated markedly nonuniform pattern of deposition within the alveolus and a strong influence of structure upon local deposition fraction. By comparing the predictions for aerosol behavior in the alveolated duct with those in an equivalent straight tube and with the results of existing experimental observation in animal studies (e.g. Zeltner *et al.*, J. Appl. Physiol., 70(3):1137–1145, 1991), the significance of structural complexity on aerosol kinetics was quantified. We conclude that the geometry of duct structure and associated flow fields have a significant role in the process of particle kinetics. (Supported by HL33009 and HL34616.)

91-80. Control of Microvascular Recruitment in the Lung: An Hypothesis

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The number of capillaries functioning within the lung is a quantity of clear importance to defining lung function. It represents the lung's vascular reserve and thus can compensate for damage or loss. Losses of capillary surface obviously impair oxygenation of the blood. Alveolar flooding is promoting when injured, leaking capillaries are recruited to vascular flow and pressure. In spite of this importance, little is known about the factors which control the extent of capillary surface under normal and abnormal conditions. Surface area is apparently controllable. Injured and hypoxic areas of the lung shunt blood flow away from the malfunctioning regions. Basic mechanics suggest that intracapillary pressure should govern recruitment, yet a good deal of variation is seen in the response of normal and injured lungs to vasoactive agents. Recent experimental results have shown that flow can control capillary surface, but that the nature of this control depends on the distribution of PVR in the lung as measured by the viscous bolus technique. These results have suggested the following hypothesis to us: Resistance distribution dictates capillary pressure and flow. It incorporates the rheological properties of blood. Flow controls functioning capillary surface, but the way in which it performs depends on the form of the resistance distribution. The lung protects itself by changing resistance distribution to minimize flow to injured areas. Drugs can affect resistance distribution through variations in arterial and venous constriction/dilation and alterations in rheological properties, perhaps including the properties of leukocytes. Therefore, we hypothesize that control of resistance distribution (not just lumped overall PVR) is equivalent to control of surface area. Therapeutic strategies should reinforce the ability of the lung to avoid injury but not affect oxygenation of systemic perfusion. The significance of this research lies in its potential to provide a rational basis for the management of capillary surface during vascular injury through the rational choice of vasoactive drugs. (Supported by P.H.S. Grant No. HL19153.)

91-81. Effect of Mechanical Strain and Fluid Flow on Endothelial Cell Metabolism

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In arterial vessels, endothelial cells are subjected to both significant periodic mechanical strain and fluid flow induced shear stress. We have examined the effect of strain (0–10% uniaxial) and fluid shear stress (0–40 dynes/cm^2) on the secretion of the arachidonic acid metabolite prostacyclin, the peptide endothelin and the protein tissue plasminogen activator (TPA) using human umbilical vein and bovine aortic endothelial cells. 10% strain at one Hertz increases prostacyclin production by over twofold, increases endothelin production 180% and has no significant effect on TPA secretion. The results are identical when cells are grown and stretched on either silastic or mitrathane (R). Arterial levels of fluid shear stress (25 dynes/cm^2) increases prostacyclin production tenfold, dramatically decreases endothelin secretion and increases TPA secretion by 300%. The differential effects are interesting and imply that the biochemical signal transduction of these two mechanical perturbations may be quite different.

91-82. Scaling of Airway Resistance Reduces Flow Heterogeneity

J.E. McNamee, Department of Physiology, University of South Carolina School of Medicine

Air flow, like blood flow, is distributed unevenly throughout the lung. Although this is partly due to gravity, other factors appear to be more important. Flow heterogeneity in a branching network is a function of each branch's resistance as well as the networks' termination conditions. Is heterogeneity also a function of how branch resistances scale from one generation to the next? Flow dispersion was calculated for mathematical models of 9-generation segments of conducting airways under the assumptions of a proportional or power-law scaling of branch resistance. To induce unequal flows, resistances in each generation were randomly chosen from Gaussian distributions whose coefficients of variation were constant. Flow heterogeneity remained nearly constant and relatively insensitive to termination conditions when resistances increased 2- to 3-fold between successive generations. If resistances were made to scale more slowly, flow heterogeneity increased and was strongly influenced by termination conditions of the network. Since the rate of change of airway size decreases down the bronchial tree, these findings imply that air flow heterogeneity increases more quickly toward the periphery of the lung. Local pulmonary ventilation may therefore be quite sensitive to alveolar events. (Sponsored in part by the Veterans Administration.)

II.10. *Respiratory Mechanics: Models of Micro-Scale Phenomena II. Tissue Mechanics*

Organizers: Jeff Fredberg and Roger D. Kamm, Harvard University and MIT

91-83. The Structural Arrangement of Collagen and Elastin Fibers in the Alveolar Entrance Ring

Robert R. Mercer, Duke University

The alveolar entrance ring is a connective tissue rich structure where the mouth of the alveolus opens into the alveolar duct. To understand its role in lung mechanics, the structure of collagen and elastin fibers in the alveolar entrance rings and the alveolar dimensions of different species (mouse, hamster, rat, rabbit, rhesus monkey, baboon and human) were studied by morphometry and serial section analysis in vascular perfusion fixed lungs. The arrangement of collagen and elastin fibers of the alveolar entrance ring was similar in all species. In serial section analysis a continuous band of elastin fibers was found to completely encircle the alveolar

mouth. Elastin fibers were frequently interwoven with collagen fibrils of adjacent collagen fibers to form a mechanical linkage between the two connective tissue elements. In the mouse, alveolar diameter and collagen fiber thickness were 58 μm and 0.34 μm respectively. In the human lung with a fourfold larger alveolus, collagen fiber thickness was increased by approximately threefold. The ratio of connective tissue fiber thickness divided by alveolar diameter, a measure of the proportionality between the pressure driving lung inflation and the resultant stresses in the alveolar entrance ring fibers, did not vary significantly between species. The results indicate that the stresses in connective tissues of the alveolar entrance ring are comparable in species with large and small alveoli.

91-84. Toward a Kinetic Theory of Connective Tissue Micromechanics

Jeffrey J. Fredberg, Srboljub M. Mijailovich, and Dimitrije Stamenovic, Harvard University, The Biomechanics Institute, and Boston University

The aim of this research is to develop unifying concepts at the level of pulmonary microstructure to account for macroscopic lung connective tissue elasticity, energy dissipation, and time-varying response to incremental mechanical loads. We establish the fiber-fiber kinetics hypothesis based upon the assumption that both rate-dependent and rate-independent dissipative stresses arise in the interaction among fibers in the connective tissue matrix. The analysis is specified in terms of geometry and material properties of connective tissue fibers and surrounding constituents. The complex three-dimensional fiber network is simplified to the interaction of two ideally elastic fibers which dissipate energy on their mutual interface surfaces; the effects of such mutual pair-wise interactions is assumed to be expressed in the aggregate. This analysis leads to the notions of the slip and the diffusion boundary layers, which become unifying concepts in understanding the mechanics that underlie both amplitude-dependence and frequency-dependence of connective tissue elasticity and dissipation during cyclic loading.

91-85. The Dodecahedron Model for Parenchymal Mechanics: Elastic and Hysteretic Moduli

Eitan Kimmel, Agricultural Engineering, Technion, Haifa

The dissipation of mechanical energy in lung parenchyma, while subjected to a periodic load, is exemplified in the incremental pressure-volume or force-displacement hysteresis loops. To account for this attribute, as well as for the elastic energy storage, the parenchyma is characterized by complex elastic moduli such as the Young's modulus $E^* = E(1 + ie)$, where E is the real modulus and e the tissual hysteresitivity. This modulus, for a given cyclic stress, $\sigma(t) = \text{Real}[\sigma_o \exp(iwt)]$ and strain $\epsilon(t) = \text{Real}[\epsilon_o \exp(iwt)]$, is defined as $E^* = \sigma_o/\epsilon_o$, where σ_o and ϵ_o denote the complex amplitudes and w is the frequency. A variational statement of non-linear structural mechanics is formulated for an individual lung element, in the shape of a regular dodecahedron. Tension members that lump together fibers and alveolar walls, form the dodecahedron edges; surface tension is incorporated to its pentagonal surfaces; and the transpulmonary pressure is simulated by an externally applied hydrostatic pressure. Tension T and length L are related, for each member, by the complex constitutive relation: $B^* = B(1 + ib) = (\delta T/T)/(\delta L/L)$, where B is the incremental stiffness and b is the member hysteresitivity. Similarly, variations of surface tension, Γ with total surface area of the dodecahedron, S, are given by $C^* = C(1 + ic) = (\delta\Gamma/\Gamma)/(\delta S/S)$. The results show how parenchymal mechanics (e.g., E and e) depend on the mechanical parameters of the microstructural elements (B, b, C and c) and on the inflating pressure.

91-86. Micromechanical Aspects of Lung Stability

Dimitrije Stamenović, Department of Biomedical Engineering, Boston University

Atelectasis is a form of instability characterized by local collapsing of lung parenchyma. It is often observed in lungs with impaired alveolar surface tension-surface area (γ–S) behavior.

The parenchyma is viewed as a prestressed elastic structure composed of a network of interconnected line elements representing the connective tissue and of a network of interconnected surface elements representing the alveolar liquid film. This description is used to study stability with respect to small disturbances. Three types of disturbance compatible with the common patterns of atelectasis are considered, focal, axial, and planar. If it were homogeneous and isotropic, then the parenchyma in situ is inherently stable. Stability is entirely provided by *any* positive force-extension dependence of the connective tissue fibers. However, if excised, the parenchyma may become unstable at low lung volumes under axial and planar disturbances. Those instabilities are enhanced by impaired γ-S behavior. If the parenchyma were inhomogeneous due to regional differences in the ratio of the alveolar surface area to lung volume, then instabilities of the focal and axial types may occur at low lung volumes. Those instabilities are also enhanced by impaired γ-S behavior. (Supported by Whitaker Foundation Grant and Grant HL 33009.)

91-87. Bulk Modulus of Normal and Emphysematous Human Lungs

Peter T. Macklem and David Bidelman, Meakins-Christie Laboratories, McGill University Clinic, Royal Victoria Hospital and Montreal Chest Hospital

We calculated specific lung elastance ($E_{S,L}$) as the change of lung elastic recoil pressure required to produce a given fractional change in lung volume ($\Delta V_L/\Delta V_L o$) as a function of transpulmonary pressure (P_L) from published data in normal lungs (N), COPD and patients with $\alpha - 1$ antitrypsin deficiency ($\alpha - 1$ AD). $E_{S,L}$ in N is the bulk modulus and was systematically greater than P_L, $dE_{S,L}/dP_L$ increased with V_L. P_L at $E_{S,L} = 30$ cmH_2O decreased with age in N but $E_{S,L}$ at $P_L = 8$ cmH_2O showed no age relationship. In both COPD and $\alpha - 1$ AD both $E_{S,L}$ and $dE_{S,L}/dP_L$ were increased compared to N. We conclude that $E_{S,L}$ is a curvilinear function of P_L in N, COPD and $\alpha - 1$ AD and is systematically greater than P_L. The increase in $E_{S,L}$ and $dE_{S,L}/dP_L$ in COPD and $\alpha - 1$ AD compared to normals probably represents two distinct abnormalities in the elastic properties of emphysematous lungs: 1) an increase in resting length of alveolar walls accounting for hyper-inflation; 2) a decrease in extensibility of alveolar walls once they become stressed. Using TLC as an index of the former and $E_{S,L}$ as an index of the latter we showed no correlation between either and FEV_L. Thus abnormalities in lung elastic properties in emphysema do not account for chronic expiratory flow limitation in emphysema. Furthermore the increased values of $E_{S,L}$ in emphysema suggest that emphysematous airspaces are poorly ventilated. As they are presumably poorly perfused, emphysema per se may not disturb ventilation perfusion ratios seriously.

91-88. Comparison of Elastic Properties of Frog Oocytes and Human Lungs

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To study the mechanical properties of single cells we measured the pressure volume relationship induced by osmotic swelling and shrinkage of a large single cell, the frog oocyte. Oocytes were surgically removed from Xenopus leavis females and placed singly on the stage of an inverted microscope with an attached video camera. The equatorial surface area of the cells was measured and, because they are spherical, cell volume (Vc), was calculated. Intracellular pressure (Pic) was obtained by micropuncture using the servo-null technique. One group of cells was placed in hypotonic buffer and allowed to swell until they burst. A second group was partially swollen in hypotonic buffer and then returned to their original volume by osmotic shrinkage in hypertonic medium. During osmotic swelling alone, Vc increased by $20.5 \pm 9.3\%$ (mean $\pm$ SD) and Pic rose to 4.11 cmH_2O (median), range 2.61–8.91 cmH_2O from a baseline of 0.27, range 0.11–0.73 cmH_2O. Near the initial volume (Vc_0) the bulk modulus ($Pic/(Vc/Vc_0)$) was 3.5 cmH_2O, range 1.8–6.5 cmH_2O and near the bursting point it was 22.2 cmH_2O, range 15.8–31.5 cmH_2O. The Pic-Vc relationship during osmotic swelling and shrinkage, revealed hysteresis. The area enclosed by the two curves normalized by $\Delta Pic \times \Delta Vc$ was 0.16 ± 0.07. These

data indicate that the bulk modulus and hysteretic properties of single cells are similar to whole lungs. Further work is needed to determine the physical entities responsible for this behaviour.

91-89. A Model of Airway Narrowing in Asthma and Chronic Obstructive Pulmonary Disease

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Exaggerated airway narrowing in response to a wide variety of nonspecific stimuli is a characteristic feature of asthma and occurs in some patients who have chronic obstructive pulmonary disease. We have developed a computational model of the human bronchial tree which allows us to investigate the effect of volume changes, airway smooth muscle shortening and airway wall thickening on airways resistance. This model is based on Weibel's symmetric lung geometry, pressure-area curves by 1–3 cmH_2O. Values of smooth muscle shortening between 20 and 40% were used in the model to generate sigmoidal shaped "dose" response curves. The analysis shows that moderate amounts of airway wall thickening, which has little effect on baseline resistance, can profoundly effect the airway narrowing caused by smooth muscle shortening – especially if the wall thickening is localized in peripheral airways. The combination of a loss of recoil and airway wall thickening are more than additive in their effect on simulated airway responsiveness. We conclude that airway wall thickening and a loss of lung recoil would explain in part the airway hyperresponsiveness characteristics of patients with chronic obstructive lung disease and asthma.

II.11. *Cardiopulmonary Engineering (General)*

91-93. Transient Ventilatory Responses of Optimal Respiratory Controllers to Exercise Disturbances

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Previous works have postulated the existence of optimization mechanisms which explain ventilatory responses to exercise. To date the transient responses of such controllers have not been examined. The present study was directed at determining the transient response implications of different controller formulations. The optimal controller of Poon (1987) was combined with a simplified dynamic model of CO_2 stores. The P_{CO_2} set-point was assumed to change with the metabolic level in an instantaneous feedforward manner in order to minimize a cost function. Even though steady state responses to exercise at different levels were consistent with a constant CO_2 level, transient changes in ventilation and CO_2 did occur in response to a step change. Model prediction was fitted with a single exponential with a time constant dependent on the metabolic rate (28–30 seconds). These time constants are shorter than experimentally measured values, thus this suggests such a direct connections is unlikely.

91-94. Application of Membrane Potential Sensitive Dye to Vascular Smooth Muscle and Heart

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Cell membrane potentials have been recorded optically in various neuronal tissues. We have developed techniques in our laboratory to obtain high sensitivity toward membrane potential changes using a "fast" voltage sensitive fluorescent (F) dye, merocyanine-540, in preparation

for applying the technique to microvessels. Here we describe methods and results obtained with vascular smooth muscle (VSM) and cardiac tissue. Cultured VSM cells were stained (5–20 mM dye in MOPS-saline) for 2 minutes and then rinsed. Longer staining increased intracellular uptake of dye giving intense nonspecific F. Images were obtained using a Lietz inverted microscope (Fluor 10×/1.25 NA) with epi-illumination (Lietz N2 filter set) and a Dage SIT camera. Signals were detected by a photomultiplier coupled to the camera port with a fiber-optic light guide mounted to an X/Y manipulator directly over the camera faceplate. A 7×7 μm^2 area was captured using a right-angle prism cemented to the end of the light guide, and thus this technique enables recording from movable sites which are localized to the camera image. Data was sampled with a 12-bit converter (Labmaster PGL) and percent changes in F, corrected for photobleaching by a linear approximation to rate of intensity loss, were determined and plotted on-line using custom software. 1 M KCl was ejected into saline and allowed to mix above the cell layer from a pipette visible to the recording camera. During KCl stimulation, F recorded from below the cell layer increased 4.6% (S/N 8:1) and returned to baseline after stimulation. Spontaneous cardiac pacemaker potentials from hamster and frog hearts stained 30–240 minutes were recorded by a similar method, although these signals may contain motion artifacts not visible in the camera image. The 8% increase in F at the peak of the action potential we recorded from heart tissue is comparable to the sensitivity obtained with RH237 styryl dye recording action potentials from cultured neuroblastoma cell monolayers (A. Grinvald et al. Biophys. J., *39*:301–308) and is 5.3 times as sensitive as previous measurements using merocyanine-540 in whole heart (G. Salmama and M. Morad, Science, *191*:485–487). This increase may be attributed to less nonspecific staining with lower dye concentration. These results indicate our method is capable of high sensitivity toward membrane potential changes, necessary to measure smaller potential shifts in microvessels. (Supported by HL12792.)

91-95. Abnormal Adhesion of Sickle Red Blood Cells to Human Microvascular Endothelial Cells: A Potential Role for the Plasma Milieu in the Initiation of Vaso-Occlusion in Sickle Cell Disease

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The abnormal adhesion of sickle red blood cells (RBC) to the post capillary endothelium is thought to contribute to the periodic vaso-occlusive episodes characteristic of sickle cell disease. Using cultured human microvascular endothelial cells (MEC) assembled into a parallel-plate model of a blood vessel, we have investigated the adhesion of sickle RBC to MEC under flow conditions which typify the shear stresses found in the post capillary venules. Results from 40 sickle patients indicate that sickle red cells are 7.5-fold more adhesive than normal red cells in the presence of autologous plasma. When suspended in ABO blood-type, Rh-factor cross matched normal versus sickle plasma, sickle RBC adhesion to MEC decreases by 69%. When sickle plasma is depleted of collagen-binding proteins, autologous plasma mediated sickle RBC adhesion to MEC decreases by 62%. These results indicate that the sickle plasma milieu is largely responsible for the abnormal adhesion of sickle RBC to MEC in our assay. Subsequent preliminary experiments indicate that sickle, but not normal, RBC adhesion to MEC is mediated by supernatant obtained from activated platelets. These results indicate that platelets may be a source of adhesive factors in sickle plasma, possibly linking the enhanced coagulation and fibrinolytic system found in sickle cell disease (Ofasu, *et al.*, FASEB, 1991, abstr.) to microvascular occlusive episodes in sickle cell disease.

91-96. Do Alternate Metabolic Pathways Exist in Oxygen Sensitive Tissues?

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O_2 metabolism has been found to be O_2-dependent in two different O_2 sensing tissues: rabbit vascular wall (abdominal aorta) and cat carotid body chemoreceptors. In the carotid body,

there is evidence for a double pathway, involving high affinity (low $K_{m,1}$) oxidative phosphorylation plus a second, low affinity (high $K_{m,2}$) pathway. A double pathway model predicts an effective Michaelis-Menten constant ($K_{m,eff}$) between the two K_m values. In vivo studies have shown that $K_{m,eff}$ decreases a few days after denervating the carotid body, perhaps due to loss of the low affinity pathway (Buerk *et al.*, J. Appl. Physiol., 67:1578–84, 1989). To examine the possible second pathway, rates of O_2 disappearance after flow interruption were measured in vitro for both tissues with recessed cathode PO_2 microelectrodes (tips < 5 μm). Experimental data were digitized by computer and fit to a 5th order polynomial spline. O_2-dependence and values for $K_{m,eff}$ were calculated from the time derivative. In the cat carotid body, 1 μg oligomycin reduced the metabolic rate and elevated $K_{m,eff}$, consistent with model predictions for inhibition of oxidative metabolism. In normal rabbit aortas, O_2-dependent metabolism was also seen, although the influence of a second pathway was not as large. In stenosis-injured walls, greater O_2-dependent metabolism was found. Other oxidases (not necessarily the same in each tissue) can explain these experimental findings. The second pathway may provide a metabolic signal which allows these tissues to sense O_2 at high tissue PO_2 levels. (Supported by HL 37048 and the Research Foundation at the Univ. of PA.)

91-97. Modelling the Fluid Dynamics of a Moving Aortic Disc-Value

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The motion of an aortic valve and the fluid flow are strongly coupled. The major characteristics are described of a two-dimensional numerical fluid-structure interaction model for the analysis of the dynamic behaviour of a disc-type prosthetic heart valve. Experiments have been performed to determine the constitutive parameters of the valve and to validate the numerical model. The experimental set-up is shortly described and a comparison is given between experimental and numerical results. The results will be elucidated of some parameter studies performed with the numerical model. The major needs for future developments will be pointed out.

91-98. Lactate Kinetics and Oxygen Delivery in Exercising Rats

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The dependence of oxygen consumption, metabolism, and exercise endurance on oxygen delivery was investigated using rats, which were chronically catheterized then progressively exercised while gas exchange was monitored and blood was sampled for analysis. The kinetics of La_a as VO_2 changed were well described by a threshold model (selected after a statistical comparison to a continuous model), and the lactate threshold (LT, the transition point where lactate begins to accumulate in the blood) occured at 55% of maximal oxygen consumption (VO_{2max}). These results indicate that, during exercise, lactate accumulation and oxygen consumption dynamics are similar in both magnitude and time course to that observed in man. The effect of exogenous lactate on endurance was investigated by continuous infusion of lactic acid to achieve elevated, steady-state arterial levels. It was found that endurance was inversely related to the rate of added lactate. In addition, there appears to be a critical lactate concentration ($\approx$ 8 mmol/l) above which, fatigue rapidly ensues. This implies that lactate may metabolically or symptomatically limit endurance capacity. Evidence also suggests that a strong link exists between lactate accumulation and oxygen availability to tissue. Oxygen delivery was manipulated experimentally by hypoxia, hyperoxia and by infusion of compounds which are thought to alter oxygen diffusivity. When oxygen delivery was increased (by any method) La_a for any given VO_2 was lower, VO_{2max} was higher and the LT occured at a greater percent of VO_{2max} than controls. When the oxygen delivery was reduced, lactate accumulation was increased and VO_{2max} reduced. The results are consistent with the hypothesis that oxygen delivery may be diffusion limited and may influence lactate production during exercise.

91-99. Regional Deformation Differences in the Costal Diaphragm: Ventral vs. Lateral

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We compared the segmental fractional dimensions (SFD) along and across muscle bundles with the regional major and minor principal stretch (PS) components of the costal diaphragm using biplane cinefluorography at 60 frame/sec. Twelve markers were implanted along muscle fibers at either the ventral (VEN) or the lateral (LAT) regions of the costal diaphragm to establish a 3 × 4 grid pattern confining six quadrilateral subregions. After surgical recovery, Mueller maneuvers were performed at five different lung volumes: FRC, 300, 600, 1,200 ml above FRC, and 300 ml below FRC. Relative movements of markers were retrieved from biplane films from which we determined SFD as well as major and minor PS components every 16.7 milliseconds. For each lung volume, deformation at the end-expiration (EE) with respect to the excised state was first determined and that at the peak inspiratory (PI) effort was related to these two states. Results show that at EE, the LAT is more stretched than the VEN region. The LAT widened by 10% at PI during Mueller maneuvers for all lung volumes, possibly caused by the flare movement of the rib cage. No significant lateral dimension changes were observed at the VEN region. At both EE and PI, the VEN and LAT costal diaphragm are under shear deformation. The LAT region is subjected to a larger shear deformation than the VEN region. Direct measurement of fiber lengths may underestimate the muscle stretching and overestimate the across-fiber dimension because of the ignored shear deformation.

91-100. Influence of Oxygen Tension on the Rheological Behavior of Sickle Cells in Microcirculation

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Experiments have shown that both elastic and viscous components in the sickled hemoglobin solution increase in magnitude due to the polymerization when the oxygen tension decreases. However, the relative contributions of the red cell membrane and the internal hemoglobin solution to total cell rheological behavior is still unknown. A mathematical model based on previously published experimental research is newly developed to study that question. The flow of sickled red blood cells in a narrow capillary is modeled by a series of deformable circular cylinders in a rigid circular tube. Each cylinder consists of an elastic membrane filled with a viscoelastic interior. The viscoelastic interior represents the properties of sickled hemoglobin solution containing both solid and fluid phases at various oxygen tensions in an average sense. The suspending plasma is assumed to be an incompressible Newtonian fluid and it may be regarded as a pulsatile Stokes flow at given frequency and mean velocity. The calculated ratio of resistance to the flow with the deformable cell at different oxygen levels to that with rigid cell is obtained. The membrane effect is included by assuming different values of membrane rigidity. The results demonstrate that cell becomes significantly less deformable when oxygen tension (PO_2) drops below 25-30 mmHg (a critical level) if the membrane rigidity is taken as a constant representative of a normal cell. The critical level of oxygen tension shifts upward if the cell membrane increases its rigidity during sickling. The study illustrates the striking dependence of sickle cell deformability on the oxygen tension during polymerization.

91-101. Two-Parameter, Resistive Pulse Analysis of Red Cell Flow Through Long Capillary Pores

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A new two-parameter analysis has been developed to determine the size and shape of individual red cells while flowing through long capillary pores from their resistive pulses. The length of the pulse plateau and the magnitude of the resistance change were analyzed to determine the velocity of the cell while flowing within the pore, the volume of the cell and its apparent diameter in the pore. In initial experiments, flow through a single capillary pore 3.2 (±.2) μm in diameter and 22.5 (±.5) μm long was studied. The analysis of apparent cell shape and the

sensitivity of the analysis to the measured and assumed parameters are detailed. The analysis was found to be robust over a range of system electrical parameters. Average cell diameter within the pore was found to be 2.82 (±.024) μm. The measured average plateau length of .98 msec produced a calculated velocity of 8.26 mm/sec. This compares to a theoretical velocity of 7.60 mm/sec. The two-dimensional parameter field of resistive pulse magnitude and plateau length was analyzed in terms of cell diameter and volume. The positive correlation between increasing pulse height and length in the red cell population was seen to be related to changes in both cell volume and the diameter of the cell within the pore.

91-102. Analysis of Blood Flow in the Dog Lung with Morphometric and Elasticity Data

R.Z. Gan and R.T. Yen, Department of Biomedical Engineering, Memphis State University

This paper presents a theoretical analysis of steady blood flow in the dog lung. Blood flow in the lung is dependent on the morphometry of the pulmonary vasculature, the elasticity of blood vessels, and the rheology of the blood. Information from those three variables has been used in our mathematical modeling of blood flow in the lung. In the model we employed, the "sheet-flow" theory is used to describe the pressure-flow relationship for the pulmonary capillaries and the "elastic tube" is used for the arteries and veins. Detailed morphometric and elastic data of the dog's pulmonary vascular system are measured in our laboratory and are applied to the model. The calculation yields the blood flow-pressure relationship of the whole lung, the longitudinal pressure distribution, and the transit time of the blood in the capillaries. The comparison of these results with the experimental results in the literature shows good agreement in many cases. (Supported by NIH NHLBI HL-34440.)

91-103. Rapid Local Evaluation of Gas Dispersion in Volume-Cycled Tube Flow

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We have developed a new method for the rapid measurement of local gas dispersion in volume-cycled tube flow. After injecting Argon tracer-gas into the oscillating flow, the time-averaged effective diffusion coefficient ($\langle D_{eff}/D_{mol} \rangle$) for axial transport is evaluated from local Argon concentration measurements taken by a mass spectrometer. Experiments were conducted in two tubes ($r = 0.85, 1.0$ cm) over a range of frequencies ($0.42 \leq f \leq 8.5$ Hz) and tidal volumes ($7 \leq V_T \leq 48$ ml.). Two methods of evaluating $\langle D_{eff}/D_{mol} \rangle$ from the concentration data are used; one uses the complete data set, while the other method uses only the local peaks of the oscillating concentration data. The experimental results show very good agreement with the theoretical predictions of transport in the absence of oscillation and during volume-cycling in the range of $4 \leq \alpha \leq 11$ and $A < 8$, where $\alpha = r(\omega/v)^{1/2}$ and $A = V_T/\pi r^3$ are the dimensionless frequency and amplitude parameters. We also show that concentration measurements taken at any radial position and any axial position within one stroke amplitude of the injection site provide similar values of $\langle D_{eff}/D_{mol} \rangle$. These methods may be applied towards measuring regional gas transport properties within the bronchial tree, and thus may be used to assess the efficacy of gas transport during non-conventional modes of ventilation. (Supported by grants from Whitaker Foundation, Parker B. Francis Foundation, American Lung Association/Rorer Pharmaceutical and NIH HL01818, HL41126, HL35440 and HL02205.)

91-104. Myocardial Contrast Two-Dimensional Echocardiography Can Be an Index of In Vivo Red Blood Cell Transit Rate

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We have previously shown the rheology of sonicated albumin microbubbles (size = 4.5 μ) to be similar to that of red blood cells (RBCs) in the microcirculation. Our hypotheses was that

the myocardial transit of these microbubbles as measured by 2D-echo would be an index of myocardial RBC transit rate in vivo. We cannulated the left anterior descending (LAD) artery in 8 dogs and perfused it with blood from the right carotid artery. LAD blood flow was varied from 13 to 110 ml/min using a roller pump. At each stage, 100 μCi of ^{99m}Technetium labeled RBCs was injected into the LAD bed and gamma emissions were sampled over the bed every 0.5 sec to generate time-activity curves. Sonicated albumin (0.5 ml) was also injected at each stage and 2D echo was performed to obtain time-intensity curves. There was an excellent correlation between actual blood flow (ml/min) and the rate of transit of both RBCs and microbubbles ($R^2 = 0.98$ and $r^2 = 0.90$, respectively). There was also a close correlation between microbubble and labeled RBC transit rate during the 39 stages analyzed ($y = 1.04x$, SEE = 0.05, $R^2 = 0.92$). We conclude that myocardial contrast echocardiography can be used to assess RBC transit rate in vivo. This offers a powerful tool for measuring regional myocardial blood flow.

91-105. Nuclear Probe Pressure–Volume Loops: A New Analysis Technique for Cardiac Hemodynamic Studies

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The nuclear probe (NP) is a non-imaging detector which provides accurate measures of relative left ventricular (LV) volume and ejection fraction (EF). The purpose of this study was to develop a small computer system for acquiring and displaying real time pressure–volume (PV) loops from NP measurements of relative LV volume. NP PV loops were acquired during a variety of interventions (infusion of nitroglycerine, passive leg elevation, ice water stimulation, coronary angioplasty, and pacing to ischemia) in 37 patients. Volume was calibrated from thermodilution cardiac output, NP measured EF, and heart rate. NP derived end diastolic volume correlated well with angiography ($r = .84$), and the PV loops shifted in the expected directions. We have demonstrated that the NP can provide safe repetitive PV loops.

91-106. Velocity Profiles Measured in a Large-Scale Model of the Human Nasal Cavity

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Velocity profiles for inspiratory and expiratory flows through a 20× enlarged scale model of the right human nasal cavity have been measured using a hot-film anemometer probe with 1 mm spatial resolution. Steady flow rates equivalent to 1260 cc/sec, 630 cc/sec, and 250 cc/sec in the real human nose were studied. From the turbulent velocity profiles, the flow distributions throughout the nasal cavity were determined, and iso-velocity contour maps constructed for various cross sections. On inspiration, turbulence generated in the external nares convects throughout the entire nasal cavity at all physiological flow rates. The average value of the intensity of turbulence has been found to be about 2.5% compared to the mean velocity. For inspiratory flows, the turbulent velocity profiles and flow distributions throughout the healthy nasal cavity are very similar for all physiologic flow rates. The average value of the mass transfer coefficient in the olfactory region has been found by momentum-mass transfer analogy to be around 0.65 cm/sec in agreement with the few other measurements in the literature. (Supported by NIH Grant #5RO1DC00072-25.)

91-107. Use of Digital Subtraction Angiography to Characterize Myocardial Intravascular and Extracellular Volumes

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We are developing a method to measure myocardial intravascular and extracellular volumes using digital subtraction angiography (DSA). The advantages over traditional methods are temporal resolution and repeatability. We first validated the method using phantoms placed in the field for DSA, along with a calibration wedge containing a solution of 1 part contrast agent (iohexol) to 6 parts water. Known amounts of this solution were added to the phantom. The volume of solution in the phantom was calculated using DSA by comparing the change in gray level in the phantom to the changes in gray level along the length of the calibration wedge af-

ter correction for scatter and veiling glare. DSA calculated volume agreed well with the known amount of contrast for volumes ranging from 5 to 40%. We have measured intravascular and extracellular volumes in isolated, perfused dog interventricular septa using different contrast agents, one of which remains intravascular and one which leaks into the interstitial space. We found values of intravascular and extracellular volumes of approximately 12 and 33 ml/100g, respectively, which agree well with published values. Being able to measure these volumes offers the opportunity to address important issues in biomechanics such as the effects of intravascular and interstitial volumes on the stress-strain properties of cardiac tissue.

91-108. Identification of Human Respiratory Mechanics Using Ventilator Driven Forced Oscillations at Low Frequencies

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Tissue viscoelasticity and mechanical inhomogeneities cause frequency (F) dependence in respiratory resistance (R_{rs}) and compliance (C_{rs}) from 0 to 2 Hz. This dependence is important to evaluate as it may be sensitive to lung disease. We compared frequency domain and time domain techniques to estimate R_{rs} and C_{rs} in four healthy adult subjects from 0 to 2 Hz and tidal volumes (V_T) from 250 to 750 ml. Data were acquired before and after endotracheal tube intubation. The frequency domain technique applies the Fast Fourier transform to sampled airway pressure and flow, while the time domain approach uses off and on-line least squares to estimate coefficients in a transfer function. Both the frequency domain and time domain techniques yielded similar R_{rs} and C_{rs} estimates implicit in a single compartment system. Breath-to-breath variations in R_{rs} and C_{rs} were usually small but in some cases substantially altered the results for all techniques. Neither method could accurately predict F dependence in R_{rs} and C_{rs} when using broad-band ventilator signals (i.e., quasi-sine or step). This may be due to V_T dependence (nonlinearities) and signal-to-noise limitations of those ventilator waveforms. Intubation results were similar suggesting little influence of the upper airways. We conclude that recursive time domain on-line tracking of R_{rs} and C_{rs} is possible, but the ability to predict F dependence in R_{rs} and C_{rs} is limited with standard ventilator waveforms. (Supported by NSF BCS 9011168, NIH HL-31248, HL-44128.)

91-109. Accuracy and Sensitivity of Continuous Blood Density Measurements for Hemodynamic Monitoring

J.R. Ligas, F. Moslehi, and M.A.F. Epstein, University of Connecticut School of Medicine

Arterial density changes induced by bolus injections of fluids into the right atrium may yield mean transit time through the central circulation and cardiac output. We formally analyzed the frequency response of the densitometer and the expected frequency components of the density signal. We used Rayleigh's method to calculate the effect of time-varying input density on vibrational frequency of the densitometer's U-tube. Fourier integral techniques were then used to determine the window associated with the instrumentation in the frequency domain. Using an approximation to dye dilution curves composed of the convolution of a distribution function representing dispersion of the bolus within the pulmonary capillary bed with an exponential decay arising from mixing in the left ventricle, we estimated the frequency components present in a density signal. Comparing this spectrum with the window determined above, we found that the densitometer's ability to accurately measure the density signal depends upon physiologic parameters (dispersion in the pulmonary capillary bed, heart rate, and ejection fraction) and upon the mechanics of the U-tube itself (flow rate, length and volume of tube). The smaller the animal and the faster its heart rate, the greater the potential for distortion in the density signal.

91-110. Reconstruction of the Antegrade and Retrograde Blood Pressure Waveforms from Flow Measurements

Mao-Zu Liu and Wei Wang, Physics Department, University of Tennessee at Chattanooga

Presented is a non-invasive approach to separating antegrade and retrograde parts of blood pressure waveforms. Blood flow data were obtained from previous in vitro experiments at three

sites along a blood vessel. A continuous pressure waveform over time is approximated from these data using a transmission line model. The antegrade and retrograde waveforms can then be resolved from one site of flow data and the characteristic impedance calculated by a simple time domain method. The reconstructed pressure waveform has good correlation with the experimental waveform, and the two resolved waveforms match the theoretical characteristics as expected, e.g. the reflected pressure and flow waves are about 180 degrees out of phase.

91-111. Measurement of Respiratory Airway-Resistance by Flow Interruption Method

H.T. Low, Y.T. Chew, T.K. Lim, and R. Chin, Department of Mechanical and Production Engineering, National University of Singapore, and *Department of Medicine, National University Hospital, Singapore*

The interrupter method, commonly used in lung ventilators, assumes that the sudden pressure change upon occlusion at the mouth is equal to the trachea-alveolar pressure-difference before occlusion. The objective of this study is to evaluate the accuracy of the interrupter method by comparison with invasive pressure-flow measurements. Also of interest will be the effect of the shutter closure-speed, breathing direction and breathing frequency. The investigation will be conducted on a simulated respiratory system based on a six-generation hollow-lung model. Measurements have been made of trachea and alveolar pressures, and flow during unassisted breathing, at 0.5 to 3 Hz, and ventilator assisted breathing, at 0.5 to 1 Hz. The pressure and flow are in phase, which supports the quasi-steady assumption. The instantaneous airway-resistance, during unassisted breathing, varies greatly between the inspiratory and expiratory phases. However, an average value could be obtained from the slope of the pressure-flow plot. The airway resistance decreases slightly at higher breathing frequency. The airway resistance obtained during ventilator-assisted breathing compares reasonably well with that from the invasive method. However, the interrupter technique shows a dependence on the shutter speed and breathing direction.

91-112. Reverse Perfused Sleeve: An Improved Device for Measurement of the Sphincteric Function of Crural Diaphragm

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A Dent sleeve device can be used for the measurement of sphincteric function of the crural diaphragm. However, in order to measure the true sphincteric pressure the diaphragm contraction has to be sustained for more than 6–8 seconds because the sleeve has a slow response rate. In a regular perfused sleeve the water flows from an oral to an aboral end. Based on the principles of sleeve function and the fact that the diaphragm moves in an aboral direction during its contraction, we predicted that reversing the flow of water in the sleeve may improve its response rate. In vitro and in vivo experiments were performed to measure the response rates of a reverse perfused sleeve. In vitro studies were done in a pressure chamber where the effect of movement of high pressure zone from an oral to an aboral end on the sleeve was determined. For in vivo experiments seven normal healthy volunteers were studied. Sphincter pressure and crural diaphragm EMG were measured simultaneously during standardized diaphragm contraction induced for 1, 2, 4 and 6 secs. Crural diaphragm EMG was measured through platinum electrodes placed on the non-pressure sensing surface of the sleeve. Standardized diaphragm contractions were induced by forced inspiration and visual feedback from the inspirometer during the inspiration. Effect of rate of perfusion on sleeve response rate was also assessed. In vitro experiments showed that the movement of high pressure zone from an oral to aboral end of the sleeve did not cause any artifact on the pressure tracing. Sleeve response rates of greater than 100 mm of Hg/sec were recorded during in vivo diaphragm contractions. The standardized diaphragmatic contractions of 1, 2, 4 and 6 seconds induced similar increases in the sphincter pressure. The rate of increase in EMG and pressure showed that there was a lag of only 0.25–0.5 secs between peak EMG and peak pressure. The rate of perfusions tested did not affect the sleeve response rates. *Conclusion*: A reverse perfused sleeve is a better device for measurement of sphincteric function of crural diaphragm.

91-113. Non-Invasive Preoperative Assessment of Cardiac Function in Vascular Surgical Patients

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Unanticipated cardiac disease plays a significant role in the postoperative complications of peripheral vascular operations. To address this, we evaluated the application of a new impedance cardiography (ICG) system of our own design. Its reliability was determined by comparing its cardiac output values (CO_{ICG}) to those obtained by thermodilution (CO_{TD}) in 22 patients from our intensive care unit ($CO_{ICG} = 1.02 \times CO_{TD} + 0.26$ r = 0.82, $p < 0.001$). The coefficient of variation was found to be 0.57, n = 21. It was then used on 40 patients whose ejection fractions (EF) had been determined by either nuclear scintography or cardiac catheterization. The percentage change in CO_{ICG} ($\Delta CO\%$) between the supine and Trendelenburg positions, a transient increase in preload, was compared to EF ($r = 0.75$, $p < 0.002$). All patients with a positive CO% had EF's > 45%; all but one patient with a negative $\Delta CO\%$ had EF's <45%. Using a new ICG system it has been possible 1) to measure CO reliably and reproducibly, 2) to distinguish patients with poor EF and therefore 3) to effectively assess preoperative vascular patients, identifying those with decreased cardiac reserve who might benefit from more intensive monitoring.

91-114. Pressure-Induced Mechanical Stress and Atherosclerosis in the Carotid Artery Bifurcation

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In this study, the connection between atherosclerosis and mechanical wall stress in arterial branch points was investigated. This study concentrated on the role of stress in the human carotid artery bifurcation. Through in vivo processes, such as MRI and ultrasound, and the in vitro studies of six human artery bifurcations obtained from cadavers, the specific geometry of the bifurcation, and the locations of the atherosclerotic lesions, were obtained. By selecting the geometrics of two representative arteries, and by utilizing the available information concerning the mechanical properties of arterial tissue, finite element representations of these specimens were created. Parametric studies varying the local and overall wall thicknesses, as well as the mechanical properties, were performed; the object being to model the normal physiological variations which occur. From results of the finite element analysis, together with the analysis of the basic shell geometry of the artery, two distinct areas of stress concentrations in the artery walls were discovered. A highly local stress concentration of approximately 9 to 14 times the proximal wall stress occurs at the point of bifurcation. A lower concentration of approximately 3 to 4 times the proximal stress occurs over a largely distributed area located at the sinus bulb. The wall thickness at the locations of these stresses have a direct effect on the magnitude, with a 50% increase in wall thickness leading to an approximate 40% decrease in the wall stress at the point of bifurcation. Comparing these results with the analysis of the six carotid specimens, a relationship between the locations of high stress and the locations of lesions was observed. Although lesions were noticed at the point of bifurcation in all the specimens, the most prominent lesions occurred at the sinus bulb. Understanding that a large area of tissue is under the effects of high wall stress at the sinus bulb, a strong correlation between distributed elevated stress and lesion formation was made.

91-115. Determination of Peripheral Airway Structure and Function from Single-Breath Gas Washout

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Single breath gas washout curves recorded at the mouth contain considerable information about the structure and function of peripheral acinar airways. We have developed a method

of parameter estimation which matches the prediction of a numerical solution of the single path convection-diffusion equation to experimental washout curves. Sensitivity studies on the numerical model have shown that total alveolar airway cross sectional area and alveolar blood-flow distribution are the main parameters affecting the slope of phase III of the washout curves while cardiac output and mixed venous blood CO_2 tension predominantly affect the height. These parameters can be easily determined by doing a least squares fit of the entire numerical and experimental curves. Use of this procedure on both patients with known airway disease and normal subjects shows that the model agrees very well with a large amount of experimental data and has the potential to become a useful pulmonary function test sensitive to early changes in the small inaccessible peripheral airways. The model is also capable of resolving several controversies and contradictions which have long been present in respiratory gas transport. (Supported by NIH Grant #RO1-HL-33891-05.)

91-116. A Theoretical Model of Gas Transport Between Arterioles and Tissue During Anemia

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A theoretical model of CO_2 and O_2 diffusion between arterioles and tissue was developed to determine if significant transport could occur in pre-capillary vessels. Since we previously found that substantial transport of CO_2 and O_2 does occur under normal physiological conditions, we decided to investigate an abnormal physiologic condition—anemia. Employing a modified Krogh structure, the model uses a finite difference scheme to solve the mass transfer equations for the arteriole-tissue system. The interactions between CO_2 and O_2 are described by the Bohr and Haldane effects and were included in the model to couple the two species together. We investigated the effects of hematocrits of 35, 25, and 15%. First, we quantified the radial and axial transport of CO_2 and O_2 in the absence of any compensating mechanism. Second, the effect on the equilibrium tissue partial pressures was assessed. Next, we examined the effect of capillary structure on the exchange. Capillary arrangements investigated included capillaries independent of the arteriole with the entering capillary PCO_2 or PO_2 equal to a constant, and capillaries branching off along the length of the arteriole with the entering capillary partial pressure equal to the arteriole partial pressure at the given axial location. Finally, we determined the compensatory increase in blood flow necessary to return tissue PO_2's to normal levels. In the smallest arteriole, we found that compared to normal conditions, PO_2 levels in the exiting blood were about 18% lower for the severest anemia, but the vessels were 12% closer to complete equilibrium with the surrounding tissue. For CO_2 the blood PCO_2 was 22% higher and the exiting blood was 55% of the way to complete equilibrium with the surrounding tissue, 10% closer than normal. Tissue PO_2 levels decreased from about 44 mmHg normally to 20 mmHg during the most serious anemia. Similarly, tissue CO_2 levels rose from 57 mmHg to 63 mmHg. For the three anemic levels studied, as hematocrit was decreased blood flow increases necessary to restore normal tissue oxygenation were 27, 89, and 231%, respectively. (Supported by NIH grant HL29737.)

91-117. A Valveless Orbital Blood Pump with No Rotating Seals

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Valves in blood pumps are expensive and provide modes of failure. Rotating seals offer sites of thrombus formation and infection. A prototype pump incorporating no valves or rotating seals was constructed and tested. In this device, fluid is pumped by the orbital action of a spiral-shaped scroll relative to an identical stationary scroll whose starting axis is rotated 180 degrees with respect to the orbiting scroll. The two scrolls, which are machined integral with scroll plates, form pockets that are filled from the outside and then ejected in the center as the orbiting scroll completes each cycle. The orbiting scroll is driven by a crank mechanism connected to a motor. Fluid is contained in the space around the scrolls by a flexible collar and does not contact the driving mechanism. The prototype pump, which is approximately 7.6 cm in diameter, 2.5 cm thick and has an orbiting radius of 5.1 mm, produced a very linear output (of water) from 4.0 L/min at 476 mmHg to 16.0 L/min at 159 mmHg at an orbital speed of 10 Hz.

At 5 Hz, flows up to 10.7 L/min and pressures up to 163 mmHg were reached and at 1.5 Hz, flows up to 4.5 L/min and pressures up to 28 mmHg. Volumetric efficiency was 50–70% at 100 mmHg depending on the speed. Several factors, such as fluid viscosity and scroll clearance and size, remain to be studied.

91-118. Perfusion Solution Protein Composition Influences Capillary Permeability to α-Lactalbumin

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The solute permeability (P_d) to α-lactalbumin in bovine serum albumin (BSA), Ringer, and frog plasma differ in the same vessel under the same hydrostatic pressure in pithed frogs (*Rana Pipiens*). In the present study, the flux of TRITC labelled α-lactalbumin in BSA, Ringer and plasma was measured under conditions of no net water flux in 12 capillaries at ΔP between 8 and 21 cmH_2O by micro-fluorometry. The mean P_d to α-lactalbumin in plasma was 0.8×10^{-6} cm/s which is less than half that measured during BSA perfusion (2.0×10^{-6}) and ten-fold lower than that measured during Ringer perfusion (10×10^{-6}). Two interpretations of these data are 1) during BSA perfusion α-lactalbumin has access to water filled routes unaccessible during plasma perfusion; 2) the capillary lumen volume accessed by the perfusion solution is modified by protein composition. The in vivo magnitude of the dye intensity on filling the vessels was a function of protein composition whereas in vitro intensity was not; thus supporting the second interpretation. (Supported by NIH HL 34872.)

91-119. Three-Dimensional Numerical Studies of the Distal Vascular Graft Anastomoses; Effects of Angle Change

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A finite element approximation of the hemodynamics of steady flow in a rigid 3-D model of the distal vascular graft anastomoses is presented. Since several authors have reported an inverse correlation between shear rate and intimal hyperplasia, the shear rates along the walls were examined in detail. The anastomoses angle was varied from 20 to 70 degrees in 10 degree increments, with 45 degrees being included as well. Reynolds numbers of 100 and 205 were investigated. Of the six regions along the walls that were studied, two were of particular interest because they exhibited low shear rates and are in areas where stenosis commonly occurs. The first of these regions (region 3) is in the outlet branch ("host" artery), downstream from the anastomoses junction on the inner wall (the wall containing the graft). The second region (region 5) is in the outlet branch, upstream from the junction, on the outer wall. It was found that by changing the anastomoses angle, the flow dynamics in these two regions could be greatly controlled. It was found that increasing the anastomoses angle increased the shear rate for region 5 but made region 3 worse. Decreasing the angle had the opposite effect on the two regions. However, decreasing from 45 to 30 degrees, the shear rate in region 3 is significantly increased with only a small decrease in region 5. It was found that a trade off existed and it is suggested that an angle of 45 degrees (with the possibility of slightly lower angles) is optimal for the anastomoses junction when considering shear rate.

91-120. Input Impedance of Pulmonary Vascular System in the Cat

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Pulmonary vascular input impedance of the cat has been studied by some investigators. However, the usual mathematical and electrical models conceived of thus far have been based on the computed data and dimensions of the pulmonary vascular bed. Also in their models the capillary blood vessels are represented by parallel circular cylindrical tubes. This is an unrealistic geometric representation of the capillary. This paper presents a model based on the detailed geometry of the pulmonary vascular bed. Arteries and veins are represented by uniform thin-walled elastic tubes with the dimensions and the elasticity of all orders measured in our laboratory. At the same time a more realistic model of capillary blood vessel of sheet-flow pro-

posed by Fung et al. is used. Pulmonary vascular input impedance is then determined and good agreement is found with experimental measurements. (Supported by NIH NHLBI HL-34440.)

91-121. Future Directions in Constitutive Formulation for Large Arteries

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Variety of constitutive formulations are proposed for arteries to describe their stress-strain behavior. This is usually done through the use of strain energy density functions. Among the forms used for the strain energy density function, the exponential and polynomial functions are the most popular ones. They are used to describe the mechanical behavior of mainly large arteries from large animals such as dogs, pigs, etc. Naturally, they are all based on variety of assumptions. Some of these assumptions are used for simplicity, the others are used because of their clinical significance and other merits. Although some of these assumptions were justified at the time, recent findings indicate that some of these assumptions are not valid and therefore the future constitutive formulations should consider these new findings which among them are the residual stress/strain, non-cylindricality of the vessel, material non-homogeneity and non-uniformity of the mechanical properties in the length and the circumferential and thickness directions.

91-122. A New Microvascular Surgical Simulator

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Most surgeons are introduced to microvascular surgical techniques on a structured course. Basic instrument handling and knot-tying techniques may, at first, be demonstrated on a piece of rubber glove material; but the conventional model used for teaching and learning microvascular anastomosis (joining blood vessels of approximately 1 mm diameter) is the rat. Human placental vessels have been used for these exercises, but they are not freely available, nor do they have a circulation. In order to simulate "reality" the rats must be anaesthetised and each student needs to use several rats during a week-long course. At the end of such a course, most surgeons do not have regular access to animal facilities where they might practice and refine the basic skills learned in the course in order to "stay current."

Until now no realistic simulator, whose vessels have the look and feel of the rat's femoral artery and vein, and which have a circulation, have been available for this training. The simulator to be presented incorporates artificial arteries and veins, each with an independent and controllable circulation. The level of realism is good and the simulators have been successfully tried and tested by surgeons on recently conducted microvascular courses taught by the author.

III. CELLULAR AND TISSUE ENGINEERING

III.1. *Biomechanics of Cell Metastasis*

Organizers: Kimberly Ward and Leonard Weiss, University of Kentucky and Roswell Park Memorial Institute, Buffalo

91-123. Deformation-Induced Destruction of Cancer Cells in the Microvasculature

Leonard Weiss, Department of Experimental Pathology, Roswell Park Cancer Institute, Buffalo

Metastasis is the most feared complication of cancer! However, at cancer cell level, hematogenous metastasis is a very efficient process, most of the cancer cells arrested in the microvascular beds of "target" organs die: *One* cause of the *rapid* death will be discussed.