Yale University School of Medicine
Thesis Abstracts — 1999

Cerebral Metabolism of Glucose and Ketones in Hyperketotic Diabetic Rat.
Chris Aiken, Gary Cline, and Gerald Shulman. Section of Endocrinology, Department of Internal Medicine, Yale University, School of Medicine, New Haven, Connecticut.

The cerebral metabolic activity of glucose and ketones was compared in ketotic rats with insulin-dependent diabetes mellitus (IDDM) and in normal controls. In the IDDM rats, ketosis had been present < 12 hours and the diabetes was of recent onset. After sufficient infusion of labeled [1-13C]glucose to achieve steady-state levels, the ratio of the fractional enrichment of the metabolites [4-13C]glutamate and [3-13C]alanine was determined by NMR spectroscopy. This ratio was higher in the normal (0.65) than in the IDDM (0.49) group (p < .005), indicating that ketones provide a significant contribution to oxidative metabolism in brain during the early phase of diabetic ketoacidosis.

Vessel Wall Activation and Remodeling after Arterial Injury: Effects of Diet and Endothelial Cell Seeding. Lee Akst, Guy VanMeter, Masashi Inaba, and Michael Conte. Department of Surgery, Division of Vascular Surgery, and Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut.

Endothelial regeneration and recruitment of inflammatory cells both occur following arterial injury and may be critical modulators of vascular healing. We hypothesized that endothelial cell seeding (ECS) might influence post-injury remodeling by down-regulation of local cellular adhesion molecule (CAM) expression in the vessel wall.

Adult New Zealand white rabbits were fed standard chow (RC, n = 4) or a cholesterol-rich diet (CF, n = 5). Animals underwent bilateral iliofemoral balloon injury, with immediate ECS using previously harvested autologous venous endothelial cells on one side and no ECS on the other (control) side. At sacrifice (1-7 weeks), vessels were harvested and sections (3/vessel) and subsequently stained with an elastin stain for morphometry. Immunohistochemical (IHC) analysis for the CAMs intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 employed specific mouse mAbs; stained sections were subsequently graded (surface, intima (I), media (M)) by two independent observers in a blinded fashion. Seeded and nonseeded vessels were compared for compartment areas (paired t-test) and IHC scores (Wilcoxon). Correlations between IHC scores and compartment areas were made using Spearman analysis.

Endothelial cell seeding reduced combined intimal-medial (I-M) area in the CF animals at 4 weeks (2.82 ± 0.4 vs. 2.08 ± 0.2 mm², p < .05). In the CF group, I-M area was positively correlated with ICAM-1 (p < .002), VCAM-1 (p < .005), and total CAM (p < .001) expression. Sections from endothelial cell seeded vessels overall demonstrated reduced ICAM-1 scores (p < .05). These results suggest that CAM expression is linked to wall thickening following arterial injury (particularly in the CF state) and that endothelial restoration may influence post-injury remodeling via this mechanism.
The Clinical Faculty of Yale University School of Medicine: The Effects of Managed Care on the Practice of Medicine. Kristin Marie Boger (Sponsored by Edwin Cadman). Department of Medicine, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study was to examine the effects of managed care on the clinical faculty of Yale University School of Medicine. Given the relative isolation of full-time faculty from the financial issues and risks associated with community practice, our hypothesis stated that the full-time faculty have been influenced less than the part-time faculty by the changes imposed by the increased importance of managed care over the years 1991 to 1996 in Connecticut. A survey was sent out to all clinical faculty members during the summer of 1996 that included questions about demographics, patient care, work habits, satisfaction, financial issues, managed care contracts, involvement in managed care and future outlook. A bivariate analysis using chi-square for categorical data and student t-tests for continuous variables was conducted. The response rate was 37 percent. Results indicated that the part-time faculty were more impacted in the areas of income, autonomy, and administrative tasks. Full-time faculty are spending less time involved in research. Although both groups still reported high levels of job satisfaction, this level was found to be decreasing. The low response rate, the use of a localized population, and incomplete demographic information are limitations of this study. Despite these limitations, this study confirms the influence that the change in payment methodology and the delivery of health care has had on physicians’ behavior and attitudes.

The Expression of CS2ST: A Novel Mouse Sialyltransferase in the Central Nervous System. Tamar Braverman, Charlene Stem, and Michael Tiemeyer. Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut.

An embryonic mouse brain cDNA library was screened to identify a vertebrate homologue of a Drosophila carbohydrate binding protein called “gliolectin,” resulting in the identification of a clone called CS2ST. The original clone was extended by 5' RACE (Rapid Amplification of cDNA Ends). Characterization of the resulting cDNA included Northern analysis and in situ hybridization.

Results of sequencing indicate that CS2ST is identical to a recently submitted sequence identified as G_{M3} synthase, an α2-3 sialyltransferase. In addition, there are areas of similarity to Drosophila gliolectin. Thirty amino acids flanking the S-motif of CS2ST are 27 percent identical and 66 percent similar to gliolectin although gliolectin does not contain an S or L motif typical of the sialyltransferases. Northern blot analysis demonstrates a dynamic pattern of expression during development with highest brain expression at stage P30 and highest liver expression at the adult stage. DNA-RNA in situ hybridization revealed staining in the cerebral cortex. RNA-RNA in situ hybridization demonstrated staining in cells of an external layer of the cortex, the mitral cells of the olfactory bulb, Purkinje cells of the cerebellum, and motor neurons of the spinal cord.

The restricted expression of CS2ST during development emphasizes the important roles of regulated carbohydrate expression in cell-cell interactions, cell substrate interactions, modulation of cell signaling, and regulation of cell growth.
Cortical Neurons Express Apoptosis-Related Proteins Variably During Normal and Hypoxic Development. Joshua Broder (Sponsored by William B. Stewart). Section of Anatomy, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Rat models of human chronic neonatal hypoxia demonstrate altered numbers of cortical neurons. We hypothesized that these altered numbers result from differential protein expression in hypoxia, disrupting normal developmental patterns of cortical neuron death. We sought to demonstrate this differential expression by immunohistochemical methods in cerebral cortex from rats raised in chronic normoxia or hypoxia.

Our study demonstrates developmental and hypoxia-induced variation in expression of several proteins. We report increased expression of the immediate early gene cellular analogue of Finkel-Biskis-Jinkins osteosarcoma virus (c-fos) in neurons of chronically hypoxic animals. Other investigators have demonstrated c-fos expression in cells fated to undergo apoptosis, suggesting that this protein may lead to reduced neuron numbers in hypoxia. We also report for the first time co-expression of vascular endothelial growth factor (vegf) and its receptors, fetal liver kinase (flk-I) and fms-like tyrosine kinase (flt-I) in both normoxic and hypoxic neurons. Binding of vegf to flk-I and flt-I in a variety of tissues inhibits apoptosis and induces mitosis; therefore, our study suggests a role of vegf in sustaining neurons during cortical development. Finally, we observe early neuronal expression of the apoptosis regulators b cell lymphoma virus gene 2 (bcl-2) and b cell lymphoma virus gene x (bcl-x) in hypoxic and normoxic animals, followed by glial expression of bcl-x in both groups. Bcl-2 inhibits apoptosis in a variety of cell types, while bcl-x modulates bcl-2 activity. These observations suggest a novel model of neuron survival in normal cortical development and under conditions of chronic hypoxia.

Predicting Child Maltreatment Using a Structured Clinical Rating Scale in the Postpartum Period. Clovne Hanchard Campbell, John M. Leventhal, Denise I. Kung, Domenic V. Cicchetti, Rachel Cohen. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut

Few studies of perinatal predictors of child maltreatment have examined the predictive validity of a screening instrument or have used the scale to structure clinical judgment to identify high-risk families. Therefore, we conducted a cohort study to test the predictive validity of the Kung-Leventhal Parenting Scale (K-L), a 22-item rating scale used to provide structure to clinical judgment in the postpartum period to identify newborns at risk of maltreatment.

During the postpartum period, clinicians completed the K-L on a consecutive sample of 363 infants who were born between November, 1989, and September, 1990, and planned to use the hospital's primary care center for pediatric care. Based on scoring criteria for inclusion in the study, 159 infants were eligible, 50 of whom were categorized as moderate/high-risk and 109 as low-risk infants. Items on the K-L were based on maternal characteristics, paternal characteristics, maternal-infant interactions, and stability of the home environment. Each item was scored by a clinician (pediatrician or social worker) on a 4-point scale from 0 to 3, and an infant was categorized as moderate/high-risk if the arithmetic sum of the scores was ≥ 9. Fifty of the no/low-risk sample were randomly selected for comparison with the moderate/high-risk group. After excluding children who had not received health care in the New Haven area, we reviewed the medical records of 92 children at four health care sites (the only two hospitals and two neighborhood health centers) from birth to the fourth birthday to determine the outcomes of maltreatment,
major changes in the child's caretaker, and growth concerns. Using predefined criteria, two clinicians, one of whom was blinded to the child's risk status, classified events as maltreatment (abuse, neglect and/or abandonment) or unintentional injuries.

Of the 92 children who met eligibility criteria for the study, 42 were classified as moderate/high risk and 47 were low risk; three were excluded because of adoption at birth or placement at birth into foster care. Maltreatment occurred in 28.6 percent of the moderate/high-risk and 8.5 percent of the no/low-risk groups (RR = 3.36, 95 percent CI = 1.17, 9.62; p = .013). Changes in the caretaker were also more frequent in the moderate/high-risk group (23.8 percent vs. 2.1 percent; RR = 11.2; 95 percent CI = 1.50, 83.78; p = .001). Differences in growth concerns were not statistically significant (9.5 percent vs. 6.4 percent; RR = 1.49, 95 percent CI = .35, 6.29; p = NS). At least one of the above major outcomes occurred in 38.1 percent of the moderate/high-risk group and 14.9 percent of the no/low-risk group (RR = 2.56, 95 percent CI = 1.17, 5.61; p = .012).

We conclude that the use of a rating scale in the postpartum period to provide structure to clinical judgment can effectively identify infants who are at high risk of maltreatment and other significant medical and social concerns.

**Characteristics of Driving Behavior Recommendations to Older Persons.**

Kerri L. Cavanaugh, Terry Z. O'Connor, Peter Charpentier, and Richard A. Marottoli. Section of Geriatrics, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut and VA Medical Center Connecticut, West Haven, Connecticut.

Two studies were performed to gain insight into the characteristics of older drivers and their relationship to recommendations given by providers and family about driving.

First, a chart review of all new patients (n = 358) seen in the Adler Geriatric Assessment Center (New Haven, Connecticut) from 7/95 to 6/96 was performed, detailing patient demographics, health status, and driving behavior. Of the patients seen, 29 percent were active drivers, 49 percent had stopped driving, and 21 percent never drove. Compared to the other groups, active drivers were significantly younger, had higher scores on cognitive and functional assessments, better distance vision, and faster gait speeds. Active drivers given recommendations (41 percent) were more likely to have a history of a crash in the past year, and lower cognitive and functional assessments. Only a minority of patients (13 percent) overtly disagreed with given recommendations.

Second, a cohort study of participants drawn from the Project Safety cohort, a probability sample of noninstitutionalized older persons in New Haven, Connecticut, was performed to identify the influence of reported driving concerns, driving-related discussions, and delivery of recommendations, in making decisions about driving patterns in older persons. Of surviving drivers in the cohort (n = 790), ages 85-95 years old, 94 percent completed the survey; 60 percent were active drivers, and 40 percent had stopped driving. The only factor significantly associated with driving concerns, discussion, or receipt of recommendations, was self-report of walking fewer blocks per day. Only 18 percent of subjects reported disagreeing with recommendations. Also, the majority of subjects reported that they felt comfortable discussing driving with their physician (78 percent), and family (66 percent).

Understanding the characteristics of drivers in older populations, factors that influence driving decision-making, and the roles of physicians and family, will facilitate the development of interventions to improve communication between patients and caregivers about the sensitive issue of driving modification or cessation.
**Developmental Expression of the Rat Rod Photoreceptor cGMP-Gated Cation Channel.** Robert K. Chiang and Colin J. Barnstable. Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut.

The appearance of cGMP-gated cation channel protein in the postnatal rat retina has been studied by fluorescence immunocytochemistry of radial retinal sections and immunoblots of retinal membrane proteins. Channel immunoreactivity was first detectable with RCNGC1-7H2 monoclonal antibody at postnatal day 7 (PN7) by both methods. Immunocytochemical label in retinal sections was localized to the outer segments, and immunoreactivity increased with increasing age. We also compared the developmental appearance of the cGMP-gated cation channel to that of other phototransduction proteins and developmental markers. RET-P2, a monoclonal antibody recognizing the 39kDa rds/peripherin disc protein, first labeled outer segments at PN7, coincident with cGMP-gated cation channel expression. Double labeling of the same section of PN7 rat retina with RET-P2 and R309 (a polyclonal antiserum against the rod cGMP-gated cation channel) revealed identical patterns of labeling. Similarly, double-labeling with RCNGC1-7H2 and an antibody against the rod cGMP-phosphodiesterase gave coincident labeling, suggesting co-ordinate expression mechanisms of phototransduction proteins with each other and with outer segment structural proteins.

**Elementary School-Age Children's Developmental Understanding of the Causes of Cancer.** Deanna G. Chin, Linda L. O’Hare, Susan T. Mayne, Peter Salovey, Donald R. Showalter, Domenic V. Cicchetti, and David J. Schonfeld. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut

This study examines children's conceptual understanding and factual knowledge of the causes of cancer. Using a standardized, developmentally-based, semistructured interview (ASK [AIDS Survey for Kids]), 784 children (43 percent black, 38 percent white, 18 percent Hispanic; 48 percent female) in kindergarten through sixth grade, attending six public elementary/middle schools in New Haven, Connecticut, were asked open-ended questions about the causes of cancer and, for comparison, the causes of colds and AIDS. Responses were scored for level of conceptual understanding and coded for factual content and factual accuracy. The level of conceptual understanding for causality of cancer increased consistently as grade level increased. When comparisons were made among the illnesses, children's level of conceptual understanding was significantly lower for the causes of cancer than for the causes of colds (p < .0001), but not significantly different from that of AIDS. Although the single most frequent cause of cancer mentioned was cigarettes/smoking (24 percent), more than one in five students stated that casual contact or contagion was a cause of cancer. More children cited casual contact/contagion than cited the following factually accurate or logically contributory causes combined: poor diet, air/water pollution or overexposure to sun, alcohol, and old age. Slightly more than one-half of students in kindergarten through sixth grade worried about getting cancer, and the vast majority (80 percent) knew that cancer could be fatal. Children have a less sophisticated conceptual understanding of cancer than of colds and a very limited factual knowledge base for cancer, and, thus, they have the capacity to increase both their understanding and knowledge. These results have implications for the creation of developmentally appropriate cancer prevention curricula for elementary school-age children.
Medicaid Managed Care in Connecticut: A Look at the Yale Preferred Health Plan. Alison L. Days (Sponsored by Edwin Cadman). Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

This thesis summarizes the history of Medicaid and managed care in this country, assesses the current Yale Medicaid Managed Care Plan through the results of a mailed questionnaire and interviews with Yale Preferred Health Plan members, and ultimately suggests several options for future changes.

A random sample of 750 Yale Preferred One (YPO) members were surveyed in July and September of 1996 concerning their opinions of the plan. Initial results highlighted several areas of concern, including transportation, the dental service, and waiting time. These results were used to focus interviews with a total of 16 patients from February to April of 1998. The data obtained were analyzed by the JMP statistical program for frequencies, percentages, and correlative data.

In analysis of the overall evaluation of the plan, 47 percent admitted that the plan system was better than Fee-For-Service (FFS) Medicaid. In addition, the frequency of Emergency Room use was decreased following enrollment in YPO (p < .01), and an increased percentage of individuals began to receive care at the Yale Primary Care Center vs. other health care centers following enrollment in YPO (p < .001).

Overall, the initial members of the Yale Preferred One Health Plan evaluated the plan highly and as a better system than what they had with FFS Medicaid. Positive aspects of being on a plan included having a primary care provider and being able to reach a provider late at night, both of which have caused the member use of the Emergency Room to decrease in frequency. Concerning aspects of transition from FFS Medicaid to YPO include problems with the dental service part of the program, transportation to and from clinics and wait time. Suggestions were made to improve these areas in the future.

Preproinsulin Is the Autoantigen Recognized by Diabetogenic CD8 T Cell Clones Isolated from Young NOD Mice. Caroline R. Dumont, F. Susan Wong, Jaana Karttunen, and Charles A. Janeway, Jr. Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut.

Insulin-dependent diabetes mellitus (IDDM) results from the autoimmune destruction of the pancreatic islet β cells. The Non-Obese-Diabetic (NOD) mouse is a murine model for spontaneous IDDM. T cells play an important role in the pathogenesis of diabetes, and CD8 T lymphocytes are critical initiators of diabetes. Insulin and glutamic acid decarboxylase have been shown to be autoantigens recognized by diabetogenic CD4 T cells; however, the autoantigens of CD8 T cells have not been previously identified. We have shown that preproinsulin is the pancreas-specific antigen targeted by highly diabetogenic Kd-specific CD8 T cell clone, G9C8, isolated from young NOD mice. The antigen was identified by screening a NOD mouse islet CDNA library using an expression cloning strategy, which takes advantage of lacZ-inducible T cell hybrids, and COS-7 cells, expressing murine Kd MHC class I molecules, as antigen-presenting cells. Pools of plasmid DNA from the CDNA library were progressively diluted. Ultimately, individual CDNA clones which stimulated the T cell hybrids were isolated and sequenced. These CDNA clones were subsequently tested for their ability to stimulate the original G9C8 CD8 T cell clones, measured by IFN-γ production as well as by ⁵¹Cr release cytotoxicity assays. These responses were confirmed to be MHC restricted as they were completely inhibited by anti-Kd antibody HB159, but not by anti-Db antibody HB51. This finding has important impli-
cations for future antigen-specific therapeutic interventions, as it has now been shown that insulin is the autoantigen recognized by both CD8 and CD4 pathogenic clones.

Macrophages, Estrogen, and the Microenvironment of Breast Cancer. Mariel Eliza, Gil Mor, and Frederick Naftolin. Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

Estrogen is a major mitogenic stimulus to established breast cancer. Estrogen sources include ovarian, extraglandular sites, and breast tissue. Which source primarily maintains benign and breast cancer tissue estrogen concentrations remains unclear. While macrophages may comprise up to 50 percent of the mass of breast carcinomas, previous studies neglected to study them as possible sources of estrogen. We present evidence that breast macrophages constitute an in situ source of estradiol and that the amount produced is sufficient to mediate cellular proliferation.

We utilized immunohistochemistry and RT-PCR to study cell-specific aromatase expression in: (i) breast biopsies, (ii) human monocytes/macrophages, and (iii) a myeloid cell line (THP-1) capable of differentiating into macrophages. Use of a breast cancer cell line (MCF-7) provided biologic confirmation of the role of aromatization in cell proliferation. Using an aromatase activity assay, we studied the effects of methyltestosterone (MT), an androgen commonly used in hormone replacement regimens, in a variety of aromatase-expressing cell lines.

We demonstrated considerable amounts of immunoreactive-aromatase (irARO) in breast tissue macrophages. Using in vitro techniques, we demonstrated that monocytes and THP-1 cells require differentiation into macrophages to produce aromatase in amounts approaching placental levels. In addition, we showed that the amount of estrogen produced by THP-1 cells stimulated MCF-7 cells to proliferate, an effect blocked by the aromatase inhibitor letrozole. We also showed significant in vitro inhibition of aromatase activity by the non-aromatizable androgen MT.

In conclusion, our studies demonstrate that estrogen is produced by differentiated macrophages in breast cancer in sufficient amounts to stimulate the proliferation of adjacent epithelial cells and to autoregulate cytokine production. These findings represent a new dimension of cellular regulation in breast tissue with major biologic implications, amenable to pharmacological manipulation.

Transient Hypertrophic Cardiomyopathy in Premature Infants. Elly V. Falzarano, Sharon E. O'Brien, William V. Tamborlane, Charles S. Kleinman, and Alan H. Friedman, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study is to examine whether iatrogenic hypertrophic cardiomyopathy (HCM) in premature infants might be induced by routine neonatal therapies including the administration of hyperalimentation, dexamethasone, and/or insulin infusion. Infants with gestational age of less than 32 weeks and birth weight less than 1250 gm were studied. Echocardiographic and metabolic analyses were performed before 48 hours of age at enrollment, and again at one, two, three, and five weeks thereafter.

Eleven patients were studied with a median gestational age of 26 weeks (range 24-29 weeks) and median birth weight of 804 gm (range 609-1230 gm). All eleven subjects received hyperalimentation, five received dexamethasone, and two received both dexamethasone and an exogenous insulin infusion. The ratio of interventricular septal to left
ventricular posterior wall thickness increased significantly from 1. 15 ± 0.06 at enrollment to 1.51 ± 0.06 (p < .05) during the third week of life, consistent with the diagnosis of HCM. This ratio returned to a normal value of 1.17 ± 0.1 by the fifth week of life. C-peptide excreted in the urine peaked during the fourth week of life at 253 ± 99 µg/g creatinine, and a peak in circulating insulin levels to 19 ± 4 µU/ml occurred during the third week of life. Both the excreted C-peptide and circulating insulin levels decreased by the end of the fifth week of life. All results are expressed as the mean ± SEM.

These preliminary data suggest that the development of a transient hypertrophic cardiomyopathy occurs in premature infants, and appears to resolve as caloric intake, insulin production, and circulating insulin levels decrease.

The Patient-Physician-Managed Care Organization Relationship: Roles, Responsibilities, and Ethics. David N. Gershfield (Sponsored by Robert J. Levine). Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The rapid growth of managed care in the United States has engendered concerns about the effects of managed care techniques on the patient-physician relationship. Traditional, dyadic models of the patient-physician relationship that maintain that physicians have a fiduciary duty toward their patients may not be compatible with the current managed care setting. A variety of models of the patient-physician relationship are reviewed, and the impact of third parties on that relationship is described. Special attention is devoted to how the influence of health insurers on the patient-physician relationship has changed over time as our health care system has moved from traditional, indemnity insurance toward the managed care practice of transferring financial risk to physicians. Managed care arrangements under which physicians assume financial risk for the medical expenses of their patients are found to be incompatible with traditional models of the patient-physician relationship. Modifications of the tripartite patient-physician-managed care organization relationship that achieve a workable assignment of roles and responsibilities to the three parties are explored.

Long-Term Psoralen and Ultraviolet A Radiation (PUVA) Treatment for Early-Stage Cutaneous T-Cell Lymphoma. Victoria L. Gross and Irwin M. Braverman. Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study is to determine whether PUVA treatment and long-term maintenance is a superior first-line treatment for early stage cutaneous T-cell lymphoma (CTCL) compared to previously used treatment modalities. 183 patient charts were reviewed and compared based on initial disease treatment. One hundred and thirty patients were determined to have T1 or T2 (early stage) disease. T1 and T2 disease is defined as less than 10 percent or ≥ 10 percent skin involvement, respectively. None of the patients had nodal involvement. Of the patients with T1 and T2 disease, 12 were initially treated with electron beam radiation alone; 66 were treated with electron beam irradiation and chemotherapy; 12 were treated with electron beam irradiation and maintenance PUVA; and 40 were treated with PUVA alone. Of the patients who received electron beam radiation alone, 58 percent were clear at the last date they were seen. The mean follow-up was 97 months (2-202). Of those who received electron beam irradiation and chemotherapy, 74 percent were clear at the date last seen. The mean follow-up was 143 months (12-275).
Of those who were treated with beam and maintenance PUVA, 75 percent were clear on the date last seen. The mean follow-up was 63 months (21-107). Of those treated solely with PUVA, 93 percent were clear on the date last seen. The mean follow-up was 66 months (9-171). Groups not initially treated with PUVA often needed PUVA salvage therapy to achieve remission. In the electron beam and chemotherapy group, PUVA salvage was only effective 66 percent of the time, suggesting that electron beam and chemotherapy might be a hindrance to future disease clearing via selection of more malignant T-cell clones. This observation and the higher relapse rate of other treatments and eventual need for salvage with PUVA strongly promote PUVA as the favored initial treatment for early stage CTCL.

**Leishmania pifanoi** Amastigote Antigen P-4: T Cell Responsiveness in Human Cutaneous Leishmaniasis. Jessica E. Haberer, Alda M. Da-Cruz, Manoel P. Oliveira-Neto, Luis Rivas, Sergio G. Coutinho, and Diane McMahon-Pratt. Department of Protozoology, Oswaldo Cruz Institute-FIOCRUZ, Rio de Janeiro, Brazil, and Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

The *Leishmania pifanoi* amastigote protein P-4 has been shown to induce significant protection against infection in mice, as well as a significant T\(_{H1}\)-like response in peripheral blood mononuclear cells (PBMC) from humans infected with *L. braziliensis*. Because a TH1-like response is associated with cure, epitope studies were conducted to further evaluate the human response to P-4. PBMC from 22 patients with cutaneous leishmaniasis due to infection with *L. braziliensis* in Rio de Janeiro, Brazil, were examined for T cell proliferation and/or cytokine production in response to whole-parasite homogenate, isolated P-4 protein and/or P-4 peptides. Twenty patients (91 percent) were found to respond to the native P-4 protein by proliferation and/or gamma interferon (IFN-\(\gamma\)) production. According to proliferation data, PBMC from 14 patients (64 percent) responded to the intact P-4 protein (stimulation index of > 2.5), and 57 percent of the P-4 responsive patients responded to at least one of the P-4 peptides. Eleven individual peptides elicited a proliferative response. Of the 17 patients examined for cytokine production, no PBMC produced detectable interleukin-4 in response to the P-4 protein or peptides. However, PBMC from 14 patients (82 percent) produced significant levels of IFN-\(\gamma\) (> 20 pg/ml) in response to intact P-4 protein, and 19 individual peptides were found to elicit an IFN-\(\gamma\) response from at least two patients. These data indicate that multiple epitopes spanning the entire P-4 molecule are responsible for the TH1-like immune response observed. The intact P-4 amastigote molecule, rather than selected peptides, may, therefore, prove to be the most useful for leishmaniasis vaccine development.

**Stole and Stethoscope: A Survey of Physician Clergy Ordained in the Episcopal Church.** Daniel Emerson Hall (Sponsored by Alan Mermann). Office of the Chaplain, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

This study presents a comprehensive census and description of physicians ordained in the Episcopal Church. The names of 230 physician clergy (83 Episcopalians) were identified by mailing inquiries to all Episcopal bishops as well as the alumni officers of medical and divinity schools in the United States. Two-thirds of the 83 Episcopal physician clergy responded to a five-page questionnaire designed to gather demographic data.
as well as narrative responses to 26 open-ended questions about the relationship between the two vocations, medicine and ministry. Extended excerpts and analysis of the responses were documented.

The studied physician clergy were a diverse group of people who defied simple categorization. Together they represented every type of medical and clerical practice. Most had attempted to integrate their dual vocations, but this integration was often only informal. Very few had trained in both disciplines simultaneously. Instead, three quarters had started their careers in medicine, and one in five had begun in ministry. The second vocation was added an average of two decades after the first.

Overall, there were two puzzling observations. First, these physician clergy struggled to articulate the theological foundations for their bivocational ministry. Second, there seemed to be little perceivable difference between the viewpoints and practice patterns of ordained and secular physicians. The author suggests two explanations: First, the church currently lacks adequate language to describe or understand bivocational ministry, resulting in widespread confusion about the role of bivocation. Second, individual physician clergy have been isolated from each other, and, therefore, they have not had a community which could hold them accountable as they struggled to develop theologies and identities as bivocational clergy.

In the hope of initiating conversation, the author presents his own theological approach to bivocational ministry; in order to facilitate dialogue within a community of accountability, the author is constructing a list-server at: www.members.tripod.com/~physicianclergy

The Use of Computer-Aided Instruction in Learning the Anatomy and Function of the Cranial Nerves. Scott W. Hines (Sponsored by William B. Stewart). Section of Gross Anatomy, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this project was to develop a computer-aided instructional (CAI) program that combines the most effective material from traditional educational resources in both gross anatomy and neuroanatomy into a single resource in order to improve both the effectiveness and efficiency of the educational process. The CAI program consists of a Web-based multimedia instructional program and three stand-alone quizzes designed to teach and test the anatomy and function of the twelve cranial nerves.

The effectiveness of the Web-based instructional program was tested in a prospective, non-randomized study comparing the performance of first-year medical students on the three quiz programs in the year prior to the release of the instructional program with the performance of students in the following year who had used the instructional program. In addition, the CAI program as a whole (Web-site and quizzes) was evaluated by responses to a questionnaire in which students were asked to rate the effectiveness and efficiency of various educational resources.

While there were not sufficient data to evaluate the effectiveness of the Web-based portion of the CAI program by student quiz performance, data from the student questionnaire showed that a statistically significant greater proportion of students gave the CAI program an effectiveness rating of ≥ 4 (good to excellent) as compared to ratings given to traditional educational resources (70.3 percent vs. 46.3 percent, p < .001). Additionally, a greater proportion of students rated the efficiency of the CAI program ≥ 4 (good to high yield) in comparison to the ratings given to traditional educational resources (66.7 percent vs. 51.1 percent, p < .10). This difference of 15.6 percent, however, was not statistically significant.
Sodium Channel Sensory Neuron Specific Messenger Ribonucleic Acid: Development in Rat Spinal Sensory Neurons. Camille M. Hylton (Sponsored by Dr. Joel A. Black and Dr. Stephen G. Waxman). Department of Neurology, Yale University School of Medicine, New Haven, Connecticut.

The sensory neuron specific (SNS) sodium channel is unique in being preferentially expressed in small dorsal root ganglia (DRG) neurons, which include nociceptive cells. We studied the developmental expression of the sodium (Na) channel alpha(α)-subunit SNS messenger ribonucleic acid (MRNA) in small (< 30 μm diameter) and large (≥ 30 μm diameter) DRG neurons using non-radioactive in situ hybridization histochemistry. α-SNS mRNA was cloned from neonatal rat DRG CDNA and produces a tetrodotoxin-resistant (TTX-R) current when expressed in Xenopus oocytes. At E17 and P2, a hybridization signal for α-SNS mRNA is not detectable in DRG neurons. α-SNS signal is present at P8, increases dramatically by P15, and appears to remain at this level in DRG neurons through adulthood. A greater percentage of small diameter, compared to large diameter, DRG neurons express moderate-to-high levels of α-SNS hybridization signal at ages ≥ P8. These observations demonstrate that α-SNS is regulated in a developmental and size-dependent manner in DRG neurons in situ. Together with previous electrophysiological studies, these results suggest that a different transcript may encode the TTX-R current at early developmental ages.

Effects of Mental Stress on Regional Myocardial Blood Flow in Patients with Coronary Artery Disease. Alexander H. Kao, Robert Soufer, Matthew Burg, Steve Pfau, James A. Arrighi. Section of Cardiovascular Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The pathogenesis of mental stress-induced myocardial ischemia in patients with coronary artery disease (CAD) is not well understood. This study examined the influence of coronary anatomy in patients with CAD on regional myocardial blood flow (MBF) during mental arithmetic. Nine patients with chronic CAD and five controls were studied using quantitative cardiac positron emission tomography (PET) with 13N ammonia during rest, mental stress (MS) consisting of mental arithmetic, and dipyridamole (dipy) infusion. All CAD patients had prior coronary angiography, and lesions were assessed quantitatively. Coronary flow reserve (CFR) was defined as the ratio of stress MBF (dipy or MS) to MBF at rest. In a separate analysis, MBF values were also normalized for rate-pressure product. Myocardial regions supplied by coronary arteries having less than 50 percent stenosis (n = 139) had decreased CFR during MS as compared to controls (p < .005). In contrast, regions with greater than 50 percent stenosis (n = 77) had similar increases in CFR with MS compared to controls (p = NS). All CAD regions had increases in CFR with dipy infusion, but the increase was less than that observed in controls (p < .001). These data suggest that blunted CFR responses to MS may occur in coronary regions with angiographically insignificant atherosclerotic disease and may be due to microvascular abnormalities.
Transforming Growth Factor-β Regulates Its Own Type I Receptor on Osteoblasts Through Changes in Nuclear Factor CBFα1. Kenneth K. Kim, Changhua Ji, Thomas L. McCarthy, and Michael Centrella. Section of Plastic Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Transforming growth factors (TGF-β) potently regulate osteoblast activity. Although osteoblasts express three conventional TGF-β receptors, signal transducing type I receptors (c) are differentially retained during osteoblast differentiation. The rat TGF-βRI gene promoter contains several cis-acting elements for nuclear factor CBFα1, whose loss is genetically associated with osteopetrosis and cleidocranial dysplasia. Because TGF-βRI rapidly re-organizes its own receptor profile on bone cells, we asked if it modified nuclear CBFα1 levels.

Primary osteoblast-enriched cultures from fetal rat calvariae were serum-deprived and treated with vehicle or TGF-βI (12-120 pM) for 24-72 hours. TGF-βRI levels were examined in nuclear-free cell extracts by Western analysis. CBFα1 levels were examined in nuclear extracts by Western analysis and by gel shift analysis with 32P-labeled DNA containing a specific CBFα1 binding sequence. Functional CBFα1 was assessed by transfecting cells with plasmid containing CBFα1 binding sequence fused to the reporter gene luciferase. Transfected cells were treated with TGF-β, and luciferase was measured.

Consistent with earlier 1251-TGF-β binding studies, TGF-β caused time- and dose-dependent decreases in TGF-βRI, with a maximal 60 percent reduction after 72 hours of exposure to 120 pM TGF-βI. CBFα1 levels were reduced in analogous ways. Consistent with these findings, TGF-β suppressed luciferase expression driven by either the TGF-βRI promoter or CBFα1 binding sequences.

Our results reveal that TGF-β down-regulates expression of its own signal transducing type I receptor. This may occur in part through a decrease in functional nuclear factor CBFα1. Loss of CBFα1 directly correlates with a decrease in bone formation. Therefore, although TGF-β is a potent osteoinductive factor, high dose or sustained exposure to TGF-β can limit its effectiveness by suppressing new TGF-βRI expression. This tightly controlled system may constitute a negative feedback loop to protect osteoblasts from TGF-β excess, and imposes important limitations needed for normal bone growth and remodeling.

Tissue Engineered Liver: Toward Form and Function. John A. Koski, Hiro Utsonomiya, Steven S. Kim, Benjamin M. Wu, Michael J. Cima, Jane Sohn, Kanae Mukai, Linda G. Griffith, and Joseph P. Vacanti. Section of Surgical Research, Department of Surgery, Children's Hospital, Harvard Medical School, Boston, Massachusetts. (Sponsored by Amy Friedman, Department of Transplant Surgery, Yale University School of Medicine).

Tissue engineering attempts to provide a solution to the donor organ shortage facing patients awaiting liver transplantation by merging cell culture with biodegradable polymers to create new constructs that replace organ function. This experiment postulates that in vivo survival of tissue-engineered liver constructs depends on maximal survival and recreation of liver-like tissue in vitro. The model in this study explores creation of an optimal in vitro culture environment to best allow hepatocytes to recreate their native structure and function by merging a new three dimensionally printed polymer design with a novel bioreactor flow system. A hepatocyte/non-parenchymal cell suspension was seeded onto three-dimensionally printed poly-lactic acid/poly-glycolic acid co-polymer constructs and
cultured for 48 hours in vitro in static culture (n = 7) or in a novel bioreactor flow system (n = 9). Constructs were analyzed using hematoxylin and cosin staining, and scanning electron microscopy (SEM). Culture media percent percent as analyzed for pH, pCO$_2$, pO$_2$, glucose, lactate, Na$^+$, K$^+$, Ca$^{2+}$, HCO$_3^-$; and assayed for albumin via ELISA. Staining and SEM analysis showed attachment and survival of cells on the novel polymer. pO$_2$ was statistically higher, and pCO$_2$ significantly lower in the bioreactor system compared to the static system (p < .05). Lactate and pH were higher in the bioreactor system (p < .1) with the pH more clinically physiologic in the bioreactor system. A trend toward higher glucose in the bioreactor system did not reach statistical significance. Albumin production was statistically higher in the bioreactor system (p < .05). This model demonstrates that a customized three-dimensionally printed polymer coupled with this novel bioreactor system may yield an in vitro environment more optimally promotes hepatocyte function compared with static culture. This experiment provides a springboard for further optimization of a controlled in vitro environment intended to support native hepatocyte function and function toward in vivo implantation as a tissue-engineered liver.

**Tamoxifen-Induced Apoptosis in Three Estrogen Receptor-Positive Human Breast Cancer Cell Lines.** Tamara Koss, Yvonne McCarey, and Kathryn D. Held. Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. (Sponsored by Sara Rockwell, Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut).

Apoptosis, or programmed cell death, is a regulated, physiological process that is central in normal development, tissue homeostasis, and the elimination of potentially damaging cells. Recently implicated in oncogenesis, apoptosis is responsible for the involution of hormone-dependent tissues such as the breast, and it has been shown to occur in a variety of tumors, both spontaneously and in response to anticancer drugs or ionizing radiation. Thus, understanding the regulatory mechanisms of apoptosis holds promise for improving cancer therapy. Apoptosis has been proposed as the mode of cell death in human breast cancer cells treated with the anti-estrogen tamoxifen both in vivo and in vitro, but previous experiments have yielded mixed results. In this study, three estrogen receptor-positive human breast cancer cell lines grown in culture were treated with different concentrations of estrogen, progesterone, and tamoxifen. None of the cell lines displayed the typical apoptotic internucleosomal degradation of DNA when analyzed via conventional gel electrophoresis. Flow cytometry analysis of propidium iodide-stained cells showed that (1) MCF-7 p53 wild-type cells underwent a significant amount of apoptosis in response to 10 μM estrogen and 10 μM progesterone; (2) MCF-7 p53 null cells underwent a significant amount of apoptosis in response to 10 μM tamoxifen; and (3) T-47D cells failed to undergo significant apoptosis in response to the three agents tested. Future work should focus on determining which cellular characteristics are responsible for the cell lines' different responses and on exploring the effects of combining hormonal treatments with ionizing radiation.
Spinal and Paraspinal B-Mode Ultrasonic Imaging, Anatomic Correlates and Findings in a Normal Cohort. Zachary R. Leitze, Volker A. Knappertz, John B. Bennett, Francis O. Walker, and Charles H. Tegeler. Department of Neurology and Department of Orthopedic Surgery, Yale University School of Medicine, New Haven, Connecticut and Department of Neurology, Wake Forest University, School of Medicine, Charlotte, North Carolina.

Prospective ultrasound imaging of the spinal and paraspinal structures in anatomical cross-sections and normal volunteers was studied to establish the anatomical landmarks in spinal and paraspinal B-mode images, to obtain normative data on imaging frequency of paraspinal structures, and to assess inter-reader reliability.

Ultrasound images of cadaveric lumbar cross-sections were measured and compared to the corresponding gross anatomical structures. Three independent investigators read paraspinal images from a normal cohort of 38 volunteers. Imaging features were analyzed for frequency and readings were compared using kappa statistics.

Ultrasound images consistently featured the osseous and soft tissue structures identified grossly with the exception of the nerve roots. The dimensions of the features in the images closely correlated to the dimensions of the corresponding anatomical structures (R = 0.95 and R-square = 0.90). Cohort images featured the spinous processes (frequency [f] = 82 percent, kappa score [k] = 0.70), the articulating surfaces (f = 80 percent, k = 0.74), the transverse processes (f = 77 percent, k = 0.85), the posterior musculature (f = 82 percent, k = 0.71), the anterior musculature (f = 68 percent, k = 0.50), the connective tissues (f = 82 percent, k = 0.58), the spinal canal (f = 40 percent, k = 0.67), the posterior curvature of the vertebral body and nerve root exit path (f = 53 percent, k = 0.68), and the nerve root (f = 4 percent, k = 0.79.)

This study reports the paraspinal features visualized and intra-reader agreement with a percutaneous posterior to anterior B-mode ultrasonic imaging technique. Further research is needed to establish clinical utilities for this technique.

Transcription Units for Immunotherapy of Melanoma. Debby A. Lin, Srinivasan Srimatkandada, and Albert B. Deisseroth. Section of Medical Oncology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Tumor cells can be specifically targeted by the action of cytotoxic T lymphocytes recognizing tumor-associated antigens and receiving co-stimulatory signals, which tumor cells may not provide. This shortcoming may be partially overcome by improving the likelihood that dendritic cells, the most potent antigen presenting cells in the body, present specific tumor antigens to T lymphocytes. In this project, we address the hypothesis that a recombinant retroviral vector encoding the melanoma-associated peptide, tyrosinase, could be produced for the purpose of genetically modifying CD34+ hematopoietic stem cells and subsequently inducing differentiation of altered cells into dendritic cells capable of presenting the antigen on the cell surface to stimulate a T cell-dependent immune response against melanoma. We succeeded in constructing the transcription units for production of pvMDR recombinant retroviral vectors encoding the tyrosinase peptide and identified growth factor regimens, which resulted in the development of dendritic characteristics in CD34+ progenitor cells. The validity of this approach in the field of cancer immunotherapy has been supported by the work of other investigators. Furthermore, other recombinant vectors containing these transcription units are being studied, with the
goal of modifying mature dendritic cells, and potentially representing a new direction in therapy for melanoma.

Characterization of Patients with Diffuse Pulmonary Arteriovenous Malformations. Yvonne W. Lui, Marie E. Faughnan, and Robert I. White, Jr. Section of Interventional Radiology, Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut.

Patients with diffuse pulmonary arteriovenous malformations (PAVMs) have involvement of multiple lung segments affecting one or more lobes. Patients with diffuse PAVMs are thought to have a poor prognosis. We attempt to characterize these patients and comment on their management and prognosis.

A retrospective chart review of all patients with diffuse PAVMs was conducted at Yale New Haven Hospital, Johns Hopkins Hospital, and St. Michael's Hospital (University of Toronto). Telephone interviews were conducted for up-to-date patient follow-up.

We present a series of 16 patients with diffuse PAVMs followed for 0.6-16.8 yr (mean 6.0 yr). At initial assessment, all patients were hypoxemic with an average $P_aO_2$ of 47.8 mm Hg, and 70 percent of patients had history of serious neurologic complication. This is approximately double the rate of neurologic complication seen in patients with discrete PAVMs. All patients in our series underwent transcatheter embolotheraphy (TCE) of large discrete PAVMs, and a few patients underwent pulmonary flow redistribution (PFR). In follow-up, three deaths occurred, one patient having died acutely after lung transplantation. Oxygenation did not improve after TCE of discrete PAVMs; however, a small improvement was seen in $P_aO_2$ after PFR ($p < .05$). Eighty-five percent of living patients are working or studying full-time.

In summary, the majority of patients with diffuse PAVMs can live with excellent function for many years. It appears that they may be at increased risk for neurologic complications especially brain abscess. Optimal treatment regimen for these patients remains unclear; however, PFR may be an option.

Prognostic Significance of Colony Stimulating Factor-1 Receptor Expression in Breast Cancer Recurrence Mary Grey Maher, Eva Sapi, Bruce Turner, Andrew Gumbs, Peter L. Perrotta, Darryl Carter, Barry M. Kacinski, and Bruce G. Haffty. Departments of Therapeutic Radiology and Pathology, Yale University School of Medicine, New Haven, Connecticut.

The macrophage colony stimulating factor receptor-1 (CSF-1R), the product of the c-fms proto-oncogene, regulates normal proliferation and differentiation of macrophages. Recent research found abnormal expression of CSF-1R in human carcinomas of the breast, ovary, and endometrium. Furthermore, activation of CSF-1R by its ligand has been shown to regulate invasiveness and anchorage independent growth in breast carcinoma cells. We designed a case-controlled study to determine the significance of CSF-1R in breast cancer. We chose 80 patients from our database of stage I and II breast cancer patients treated with conservative surgery and radiation therapy (CS+RT). Expression of CSF-1R in the tumors of 40 patients who experienced an ipsilateral breast tumor recurrence (IBTR) as a primary site of relapse were compared to 40 patients who had not experienced an IBTR. The index and control patients were matched by age, clinical stage, nodal status, and follow-up. Paraffin-embedded sections were immunostained with antibodies directed toward
CSF-1R. For the CSF-1R antibody, a total of 28 index cases (70 percent) demonstrated strong staining, while only 16 control cases (40 percent) demonstrated high immunoreactivity (p = .007). The CSF-1R antibody showed a positive correlation for local relapse. In summary, our findings provide evidence for the poor prognostic role of CSF-1R in IBTR.

Gender Differences in Medical School Debt Among Yale Medical Students in the Classes of 1990-1999. JoAnne M. McDonough (Sponsored by Nora Groce). Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

This thesis investigates the presence of gender differences in medical school debt among Yale medical students graduating between 1990-1999, and if present, to investigate what factors account for this difference. Data from 903 students who applied for financial aid were analyzed for a difference in mean educational debt, and multiple regression techniques were used to adjust for factors that may account for the gender difference. Both medical school tuition and debt are increasing ahead of inflation. 70.9 percent of students had medical school debt. Of these, the mean medical school debt was $68,396. Women had significantly higher medical school debt, levels of need-based loans, and higher adjusted calculated parental contributions. The gender difference in debt disappeared when adjusted for years of matriculation and graduation, as well as parental contribution. 58.1 percent of all students, and 74.8 percent of students with debt, submitted parental information. Of the debtors, an average of 103.4 percent of the calculated parental contribution was met with market rate loans. It appears as though the gender difference in medical school debt is a result of 1) more the increasing number of women medical students in recent years, when debt has increased, and 2) higher calculated parental contributions not being met by students' families.

Evaluation of a Human-Murine Xenogeneic Hematopoietic Stem Cell Transplantation Model. Matthew E. Mealiffe and Diane S. Krause. Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut.

Human hematopoietic stem cells are responsible for the long-term multilineage engraftment seen in clinical stem cell transplantation. Human hematopoietic cells can produce long-term, multilineage engraftment in sublethally irradiated immunodeficient mice. The three aims of the present study were: 1) to characterize the engraftment of human peripheral blood stem cells (PBSCS) and cord blood (CB) in NOD/SCID mice; 2) to determine the contribution of human species-specific growth factors to the xenogeneic engraftment of human PBSCS; and 3) to induce human megakaryopoiesis in vivo in the NOD/SCID mice receiving human CB transplants by the administration of human megakaryocyte growth and development factor (hMGDF) and human interleukin-3 (hIL-3).

Human CD34+ PBSCs engrafted four of 12 sublethally irradiated NOD/SCID mice analyzed 10 weeks post-transplant (bone marrow human DNA content = 0.06-1.3 percent). Five of 18 analyzable mice transplanted with human cord blood had measurable levels of human cells in their bone marrow at four weeks post-transplant (human bone marrow cells = 1.8-13.8 percent). No megakaryocytopoiesis could be observed immunophenotypically by flow cytometry in the cord blood transplanted mice treated with hIL-3 and/or HMGDF. In contrast, bone marrow from the hMGDF-treated mice yielded CFU-MK in vitro (a more
sensitive assay for megakaryocytic progenitors) whereas bone marrow from untreated mice did not.

In conclusion, although both human CB and CD34+ PBSCs engraft the bone marrow of NOD/SCID mice, cord blood more readily engrafts. Furthermore, HMGDF can support the proliferation and maintenance of CFU-MK in NOD/SCID mice transplanted with human CB. Additional studies will be necessary to identify factors associated with higher, more reproducible levels of engraftment and to determine if there is correlation between human stem cell behavior in vivo in the human microenvironment and in the immunodeficient murine microenvironment.

Factors Which Influence the Decision to Hospitalize Children with Acute Asthma. Jason L. Merritt, Frances S. Shofer, and Jill M. Baren. Section of Emergency Medicine, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Despite the existence of expert panel guidelines that recommend the use of objective measures of lung function in the assessment of acute asthma, there is evidence of poor physician compliance. Our goal was to determine how physicians make disposition decisions concerning pediatric asthma patients in the emergency department (ED), specifically the use of subjective vs. objective data. This prospective observational study of a cohort of physicians in an urban pediatric ED examined 31 factors that may have influenced the disposition decision for patients presenting with acute asthma. These factors included pre- and post-treatment (pretx, postx) values, baseline disease characteristics, social variables, and clinician judgment. Physicians completed a questionnaire at the time each disposition decision was made, and the data obtained were analyzed using a general estimating equation to control for clustering of individual decisions, comparing factors used for admission vs. discharge. Fifty-six physicians completed forms on 313 of 362 consecutive asthma patients (86 percent), of which 103 (33 percent) were admitted. Factors used more often for admission included two objective (pretx respiratory rate, 54 percent vs. 38 percent; postx oxygen saturation, 60 percent vs. 24 percent; p < .01 for both) and three subjective (pretx dyspnea, 49 percent vs. 27 percent; prior hospitalizations, 28 percent vs. 18 percent; "gut feeling," 39 percent vs. 21 percent. p < .01 for all). Factors used more often for discharge included six subjective (pretx color, 7 percent vs. 45 percent; prior intubation, 7 percent vs. 22 percent; steroid use, 14 percent vs. 24 percent; home equipment, 9 percent vs. 53 percent; follow-up, 2 percent vs. 49 percent; medication compliance, 2 percent vs. 46 percent; p < .01 for all). Objective factors were not used by 20 physicians (36 percent) in 47 (15 percent) patient decisions. Peak flow rate and pulsus paradoxus did not contribute to decision making. We conclude that physicians often rely on subjective criteria when making disposition decisions, and, therefore, the implementation of asthma guidelines may be hindered by actual physician practices.

Prefrontal Cortical Dopamine and Its Cognitive Correlates. Beth L. Murphy, Amy F.T. Arnsten, and Robert H Roth. Departments of Pharmacology and Neurobiology, Yale University School of Medicine. New Haven, Connecticut.

Despite numerous studies of the biochemical sensitivity of the prefrontal cortical (PFC) dopaminergic (DA) system to stress and the involvement of DA in the neural basis of PFC-dependent cognitive functions, the effect of increased DA turnover on PFC cognitive functions was unknown. The anxiogenic, FG7142, produces a PFC-selective
increase in DA turnover in rodents, as measured by whole-tissue DA and its metabolite (DOPAC). When FG7142 is administered to rodents and non-human primates, they show impairment on PFC-, but not non-PFC-, dependent tasks. The FG7142-associated cognitive impairment in both rats and monkeys can be prevented by pretreatment with the benzodiazepine antagonist, RO151788, consistent with action of FG7142 at the benzodiazepine/GABA<sub>Α</sub> receptor. The noradrenergic α<sub>2</sub>-agonist clonidine and the glycine/NMMA partial agonist (+)HA-966 prevent the FG7142-induced increase in DA turnover in the rodent PFC. In addition, the microinfusion of (+)HA-966 directly in the ventral tegmental area (where DA cell bodies that project to the PFC are located) prevents the FG7142-induced increase in DA turnover. When administered as pretreatments, both clonidine and (+)HA-966 ameliorate FG7142-associated cognitive impairment in spatial working memory in the rat and monkey. The direct blockade of DA receptors was examined by using DA antagonists. Both typical and atypical antipsychotics (haloperidol and clozapine), ameliorated FG7142-associated delayed alternation and delayed response performance deficits in rats and monkeys. In addition, both D<sub>1</sub> and D<sub>2</sub>-receptor family antagonists (SCH39166, SCH23390, and sulpiride) were effective in preventing FG7142-induced PFC-dependent cognitive deficits. These studies demonstrate that 1) increased DA turnover in the PFC is associated with PFC-dependent cognitive deficits; 2) the ventral tegmental area plays an important role in regulating PFC DA; 3) both the D<sub>1</sub> and D<sub>2</sub>-receptor families can modulate PFC-dependent cognition. These studies have direct implications for the understanding and treatment of psychopathology involving DA systems in the PFC, as well as normal stress-perturbed cognition.

The Reliability, Diagnostic Efficacy, and Validity of the Millon Adolescent Clinical Inventory. Michelle M. Pinto and Carlos M. Grilo. Department of Psychiatry, Yale Psychiatric Institute, Yale University School of Medicine, New Haven, Connecticut.

The Millon Adolescent Clinical Inventory (MACI) was designed for mental health professionals to aid in identifying and understanding a wide range of psychological difficulties in adolescents. While the MACI's pragmatic clinical significance has been noted in a number of recent psychological assessment books, little research has focused on its psychometric qualities. This study examined the internal consistency reliability, diagnostic efficacy, and discriminant validity of selected scales of the MACI. A series of 322 psychiatrally hospitalized adolescents was administered the MACI, and a battery of psychometrically well-established self-report measures soon after admission and a multidisciplinary team independently assigned Diagnostic and Statistical Manual of Mental Disorders (3rd edition, revised) diagnoses at the time of discharge. The internal consistency of MACI scales ranged from very good to excellent. The MACI demonstrated good diagnostic efficacy for most prevalent psychiatric disorders but was less useful for detecting certain low frequency disorders. The MACI showed good discriminant validity for most disorders, with subjects with a clinically-derived diagnosis having a significantly higher corresponding MACI scale score than subjects not assigned that diagnosis. Concurrent validity, tested by correlating MACI scale scores with those of relevant, validating measures, was generally good. The MACI appears to be a psychometrically sound self-report instrument, providing useful diagnostic information and appearing most helpful as a screening instrument for many problems found in adolescent psychiatric inpatients.
Medicine and Motherhood: Shifting Trends Among Female Physicians from 1922-1999 at Yale University. Ruth A. Potee and Jeannette R. Ickovics (Sponsored by Michele Barry). Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Rising numbers of women in medicine, changing roles of men within the family, and alterations in the delivery of healthcare continue to shape the interaction between medicine and motherhood. The objective of this study was to examine patterns of work and family among female physicians over the past 80 years.

A Questionnaire was mailed to all female matriculants to Yale University School of Medicine from 1922 to 1999 (n = 863). The survey included questions regarding personal and professional demographics, career satisfaction, child-rearing, childbearing, and role-conflict assessments.

The average age of female medical school matriculants has increased over the last 80 years. Eighty-two percent of women over 40 were mothers and 18 percent were not. Half of those with children had their first child prior to the completion of medical training. The amount of time taken by women for maternity leave has increased over the last eight decades, although the level of satisfaction with length of leave has dropped. On average, 1.8 providers, in addition to the mother, cared for the children for ten or more hours each week. Female physicians without children were more likely to be in surgical specialties, less likely to be in primary care, and more likely to work full-time than their female colleagues with children. Two-thirds of women with children believe that being a mother has slowed their career progress.

The conflict between parenting and doctoring arises earlier in medical training for graduates in the latter half of this century. The rigidity of medical school and residency training is in contrast to the relative flexibility of the practice of medicine, at least outside academia. We conclude that more changes that place greater emphasis on the importance of caring for one's own family as a physician are necessary in the training of doctors and practice of medicine.

In the Eye of the Patent Holder: Dr. Samuel Pallin and the Chevron Incision. Badrinath Rengarajan (Sponsored by Frederic L. Holmes). Section of the History of Medicine, Yale University School of Medicine, New Haven, Connecticut.

This project describes the controversy surrounding the patenting of medical and surgical methods by telling the story of Dr. Samuel Pallin's 1993 lawsuit against fellow ophthalmologist Dr. Jack Singer for infringing the patent on his chevron sutureless incision technique. This project seeks to comment on the effects of Dr. Pallin's lawsuit on his patent and on the medical profession, to understand the basis for banning medical procedure patents (or their enforcement), and to understand why participants in this controversy behaved as they did. Methods included interviewing key participants and examining historical literature, legal articles, court documents, and medical texts.

Pallin sued Singer because Singer's incision technique was most similar to his own work, and a successful suit against Singer would clear the path to enforce his patent against other physicians. However, the sutureless incision techniques of other surgeons predated Pallin's patented work, and the chevron incision technique is rendered obvious to an ordinary ocular surgeon when one takes into account the full body of state-of-the-art knowledge at the time Pallin claims to have invented his method. Thus, his patent was effectively invalidated. The AMA and other medical societies feared that patents on medical methods would lower the quality of patient care, compromise physician autonomy
and the doctor-patient relationship, hinder the dissemination of knowledge, and increase health care costs and litigation. They lobbied Congress to ban the patenting of medical methods while continuing to allow the patenting of medical products. In 1996, perhaps to placate the medical community, Congress banned the enforcement of medical method patents against health care providers. However, the concerns of the opponents of medical method patents could have been allayed with changes in practices at the U.S. Patent and Trademark Office. Although Pallin's lawsuit against Singer informs our understanding of other medical method patent controversies, medical method patents are not likely to be a significant problem.

**Reduction Ventriculoplasty for Dilated Cardiomyopathy: The Batista Procedure.** Shahram Salemy, George Tellides, and John A. Elefteriades. Section of Cardiothoracic Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Approximately 50 percent of individuals with advanced dilated cardiomyopathy die within one year of onset. Reduction ventriculoplasty (Batista procedure) has been proposed as a single discrete intervention that can restore normal cardiac chamber size and function in these patients. We investigated this experimental procedure in patients who were not candidates for the conventional option of cardiac transplantation. Nine patients (eight male, one female) with advanced dilated cardiomyopathy underwent reduction ventriculoplasty at our institution from January, 1996, to August, 1997. Mean age was 53 years (range 36-75), and patients were New York Heart Association (NYHA) functional class III (2) or IV (7). Transplantation was not indicated due to patient preference (4), advanced age (2), alcoholism (1), morbid obesity, and pulmonary hypertension (1), and bronchopleural fistula with bilateral empyemas (1). Reduction ventriculoplasty was performed by anterior (2) or lateral (7) myocardial resection in conjunction with tricuspid repair (4) or mitral repair (7). Three patients died after 2 days, 11 days, and 5 months, respectively, due to heart failure. Mechanical and pharmacological support was weaned between 0-4 days in seven patients, and one remained dobutamine dependent for 34 days. Patients were discharged home on postoperative day 5-43 (median = 13). The six surviving patients are in NYHA functional class II (4) and III (2). Serial echocardiography revealed an increase in left ventricular ejection fraction from 15.3 percent preoperative to 32.5 percent at 1 year post-reduction (p < .0005). Multiple-gated acquisition scanning showed modest decrease in left ventricular end-diastolic volume from 394 ml preoperative to 328.5 ml post-reduction (p < .3). Reduction ventriculoplasty improves cardiac size and function with acceptable operative mortality and early survival in high-risk patients with non-ischemic dilated cardiomyopathy who are not transplantation candidates.

**Informed Consent in the Pediatric Emergency Department: Parent Perceptions.** Michelle S. Sanders, David Schonfeld, Frances S. Shofer*, and Jill Baren'. Section of Emergency Medicine, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut. (*Currently at Children's Hospital, Philadelphia, Pennsylvania.)

In this pilot study, we wished to determine whether for simple procedures performed in the pediatric emergency department parents could demonstrate having received, understood, and processed the basic elements of informed consent described in the literature. We conducted a series of 30 semi-structured interviews of parents whose children had
recently undergone lumbar puncture, fracture reduction, or suture repair of laceration. We found that many parents could not demonstrate having had risks, benefits, and alternatives explained during the informed consent discussion, although many parents felt involved in decision-making nonetheless. Parents of children undergoing diagnostic procedures (lumbar puncture) felt significantly more pressured and significantly less comfortable with their clinicians' explanations than did parents of children undergoing therapeutic procedures (fracture reduction and suture repair). Parents with prior experience with the procedure in question felt significantly more comfortable with their clinicians' explanations than did parents without such prior experience. Clinicians need to be aware that the pediatric emergency department is a unique setting in which parents of children with diverse illnesses undergoing diverse procedures are subject to diverse stressors that can interfere with information exchange during informed consent discussions.

Managing Childhood Seizures with Antiepileptic Drugs in the Emergency Department. Sherri Denise Sandifer (In collaboration with Karen Schneider and sponsored by M. Douglas Baker). Section of Emergency Medicine, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Pediatric seizures account for more than 300 visits each year to the Yale Pediatric Emergency Department. With the exception of simple febrile seizures, which usually do not require treatment with antiepileptic drugs (AEDs), pediatric seizures are often managed pharmacologically. Despite the development of new “safer” antiepileptics with a shorter onset and duration of action, the use of those agents continues to carry the risk of complications such as respiratory depression, which can result in significant patient morbidity.

This retrospective chart analysis examined 1) the frequency and appropriateness of use of antiepileptic drugs in the management of acute pediatric seizures in the emergency department and 2) the relationship of antiepileptic drug use with disposition of patients from the pediatric emergency department.

Between April 1, 1996, and March 31, 1998, 739 children presented to the Yale Pediatric Emergency Department (PED) with acute seizures. Of those, 404 children had simple febrile seizures, received no antiepileptic drugs, and were excluded from analysis. Of the remaining 335, 318 were initially treated in the Yale PED, with 227 (71 percent) discharged home, 73 (23 percent) admitted to the inpatient ward (IW), and 18 (6 percent) admitted to the PICU. Another 17 patients were initially treated in another PED and then transported to Yale for further evaluation.

Children receiving two or more AEDs were more likely to be subsequently hospitalized (46 of 68, 68 percent) than those receiving only 1 AED (17 of 58, 29 percent). Children admitted to the PICU also tended to be more likely to have received two or more AEDs than those admitted to the IW (89 percent vs. 43 percent). Forty-one children who had active seizures at the time of their first AED received lorazepam as their first AED. Of those, 13 children (32 percent) received a second AED within 8 minutes of the first lorazepam dose. Twenty-five children received 29 back-to-back doses of lorazepam. In 16 of those instances (55 percent) the second lorazepam dose was given before the onset of action of the first dose.

These data indicate that AEDs are commonly incorporated into PED management of seizures. In the Yale PED, lorazepam is the most commonly used benzodiazepine. Doses of lorazepam are often given back-to-back, often before the first dose has been given an adequate opportunity to work. This factor appears to influence subsequent management and disposition of these children.
The Ductus Venosus in Premature Infants. Sandra L. Santiago and Alan H. Friedman. Section of Cardiology, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Although well studied in animal models and in humans, no studies address the ductus venosus (DV) in the preterm neonate. The purpose of this study was to assess the timing of postnatal closure of the DV in premature infants compared to full-term neonates and to determine if a correlation exists between patency of the DV and patency of the ductus arteriosus (DA) in this population. We enrolled ten full-term infants and twelve preterm infants in a prospective, non-invasive study to test the hypothesis that the DV remains patent longer in the preterm infant compared to the term infant, and that patency of the DV is associated with patency of the DA. Sonographic evaluation of the DV and DA was performed in premature and full-term infants in the first three days of life and repeated weekly until closure was documented. Clinical data were analyzed daily. The DV underwent early closure (before the third day of life) in seven of the 12 (58 percent) preterm infants and in none of the term infants \( p = .01 \). Closure of the DV was documented occurring a median of 11 days after birth (range 8-17) in the preterm infants with late closure (after day of life 3) and 13 days (range 8-15) in the full-term neonates. The clinical variables discussed as possible explanations for early closure of the DV in premature infants include indomethacin administration, hypotension, and lack of enteral nutrition in the first days of life. No statistically significant association was found between patency of the DV and patency of the DA in premature infants. We conclude that the DV closes earlier in premature infants than in full-term neonates, a previously unreported phenomenon. The mechanism of early closure of the DV in premature infants remains speculative.

The Impact of Medicaid Managed Care on the Lives of the Chronically Mentally Ill in San Francisco. Deborah P. Steinbaum (Sponsored by Mark Schlesinger). Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

The changing treatment patterns of 154 severely mentally ill individuals were tracked over a five-year period just before and during San Francisco's transition to Medicaid managed care to determine the effects of implementation of Medicaid managed care on a chronically mentally ill population. Sources of information included INSYST (a billing/records database), "Bed Committee" records, and departmental memos and reports. Tests of statistical significance were conducted using an independent sampling design.

The proportion of clients using acute services decreased from 65.9 percent in 1994 to 41.8 percent in 1998, and the proportion of clients in semi-permanent care increased from 11.0 percent in 1994 to 52.5 percent in 1998, indicating that managed care is shifting the San Francisco mental health system's focus from crisis management to long-term, chronic care. Both of these changes were statistically significant \( p < .000 \) as was a decrease in the proportion of clients using intermediate level services was also statistically significant. By 1998, significantly higher proportions of clients in assertive community treatment (ACT) plans used acute and intermediate level services than did their non-ACT counterparts; thus, even with an ACT intervention, they were less able to maintain stability in the community. Substance abusers used similar amounts of care when compared to non-users except in the last year of the study, when they were significantly
less likely to need acute care. This was largely due to a decrease in emergency room usage, which may represent the beginning of a shift in patient attitudes.

Evaluation of T-Cell Receptor Gene Rearrangements in Patients with Recurrent T2 CTCL (Mycosis Fungoides). Meena Thayu, Earl J. Glusac, Barry M. Kacinski, Giovanni Tallini, and Lynn D. Wilson. Department of Pathology, Department of Dermatology, Section of Dermatopathology, and Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut.

Cutaneous T-cell lymphoma is a clonal neoplasm of epidermotropic CD4+ T lymphocytes, which includes the entity mycosis fungoides (MF). After identification of patients with recurrent MF treated with total skin electron beam therapy (TSEBT) at the Yale University School of Medicine, this study attempted to compare T-cell receptor (TCR) γ gene rearrangements via polymerase chain reaction (PCR) in both original and recurrent skin biopsies from these patients. Between 1974 and 1996, a total of 95 T2 MF patients were treated with TSEBT, and four of these were identified for the study. Slides and tissue samples of both primary and recurrent skin biopsies for each patient were confirmed as being consistent with CTCL. DNA for PCR was isolated from paraffin embedded tissue samples. Using consensus primers, which hybridize with conserved regions of the TCR gene, these regions of the genome were amplified. The PCR products were then analyzed by acrylamide gel electrophoresis. Of the primary and recurrent samples from four patients with a median DFI of 1222 days, only two showed evidence of a dominant TCR clone. A number of factors, including lack of sequence homology between the primers and the gene segments, the existence of multiple neoplastic cell lines, DNA degradation in the archival samples, and the presence of reactive as well as malignant lymphocytes, may have prevented the detection of dominant TCR rearranged clones in the samples. Despite the results of this study, TCR analysis via PCR and gel electrophoresis continues to be of utility in the evaluation of patients with MF when used in conjunction with other diagnostic modalities and in cases with nonspecific clinical, histopathological, and immunophenotyping findings.

The Role of White Yams in the Increased Incidence of Multiple Births in Southwestern Nigeria. Obi Ugwonali, Friday Okonofua, Kunle Odunsi, James Jekel, Grace Wyshak, and Frederick Naftolin, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

Multiple pregnancy is a major cause of prematurity and other perinatal complications. It has been reported that the Yoruba people of southwestern Nigeria have the highest incidence of multiple births in the world, resulting in perinatal mortality rates more than 10-fold greater than that in the United States. Therefore, it is important to find out what factors play a role in this high incidence of multiple births in southwestern Nigeria.

Study 1: A multicenter demographic survey was analyzed to generate a current incidence and relationship between socioeconomic factors and twinning. Of 6,414 deliveries, there were 267 twin pairs and nine triplets giving an incidence of 41.6 per 1000 and 1.4 per 1000 births for twins and triplets, respectively. There was also an inverse relationship between twinning and socioeconomic status.

Study 2: An age-matched case control study of 254 twin and singleton mothers was carried out in two hospitals in Ife, Osun State. This survey elicited information on the
biosocial profile of the women and their husbands and their dietary habits. There was a three-fold increase in risk associated with yam consumption (p < .05) as well as a direct dose-response relationship between reported yam (Dioscorea rotunda) consumption and twinning (p = .001). Again, there was an inverse dose-response relationship between socioeconomic status and multiple births (p = .009).

Study 3: Rat experiments were conducted to determine the effects of yams on litter size, uterine weight, and number of oocytes ovulated during estrus in order to determine its mechanism of action. Yam-fed rats had larger litter sizes (p = .02), higher pregnancy rate (p = .03), higher number of oocytes during estrus (p = .07) and a greater inhibition of uterine weight gain induced by estradiol (p = .003) when compared to control rats.

These data confirm that multiple births continue with high incidence in southwestern Nigeria, especially among Yoruba women of lower socioeconomic status and that yam consumption correlates with an increased risk of multiple births. Furthermore, the rat studies suggest that Nigerian white yams contain antiestrogens that can cause an increased ovulation rate in rats and humans. However, it remains to be determined the exact nature of these compounds and what the genetic contributions to this picture are.

Film Documentary as Ethnography: Toward A New Medical Ethics. Angelo Volandes and Robert J. Levine. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Analytic philosophy has been the dominant force in shaping the young field of medical ethics in this country. Analytic philosophy's monolithic voice has often drowned out the voices of other disciplines that could potentially contribute to medical ethics. This thesis is essentially a critique of the sole use of analytic philosophy in framing and teaching theories of medical ethics.

After reviewing numerous critiques of a medical ethics based only in analytic philosophy, the ethnographic approach toward medical ethics will be described. Ethnography, and, more specifically, film documentaries, offer a novel alternative toward medical ethics. The ethnographic and film lenses begin the analysis from the lived details of individual clinical cases as opposed to analytic philosophy's stance from the abstract temple of reason. Both written and filmed ethnographies will be used to argue for an ethnographic presence in medical ethics.

Finally, an original film documentary titled Illness As Experience will take the viewer into the world of a severely disfigured patient and will serve as a powerful example of the ethnographic shift that this thesis argues for. Whether through the lens of a camera or through the eyes of an observant ethnographer, film documentary as ethnography has a powerful place in the burgeoning field of medical ethics.

Coronary Revascularization Restores Hibernating Myocardium in Advanced Ischemic Cardiomyopathy. Meher Yepremyan and John A. Elefteriades. Section of Cardiothoracic Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The study evaluated the last 12 years of isolated coronary artery bypass surgery (CABG) performed at Yale in patients with severe left ventricular (LV) dysfunction and identified the candidates who may benefit from coronary revascularization despite eligibility for transplantation. The recruitment of hibernating myocardium underlies the improvement in EF and in symptomatic state consequent to CABG. The protection of
viable myocardium from incremental infarction underlies the improvement in survival. The study analyzed the data of 189 consecutive patients (157 men, 32 women, aged 42 to 83 years [mean: 67]) with a LV ejection fraction (EF) of less than 30 percent (mean: 23.3 percent) who underwent isolated CABG performed by one surgeon during a 12-year period. Preoperatively, 69 percent of patients had angina, 66 percent had congestive heart failure (22 percent with pulmonary edema), and 22 percent manifested significant ventricular arrhythmia. The hospital mortality rate was 5.3 percent (only 2.8 percent for those who did not require admission to an intensive care unit immediately before operation). Mean follow-up of 40.5 months revealed Canadian Cardiovascular Society angina class improvement postoperatively by 2.1 categories and New York Heart Association congestive heart failure class by 1.7 category. The LVEF improved from 23.3 percent preoperatively, to 33.3 percent postoperatively (p < .001). The late postoperative EF redetermination at a mean of 60.4 months postoperatively increased from 23.5 percent preoperatively to 30.4 percent at late follow-up. At three, five, and seven years, all-cause survival (including hospital deaths) was 75 percent, 59 percent, and 57 percent, respectively. In patients with coronary artery disease and advanced ventricular dysfunction: 1) CABG can be performed relatively safely; 2) left ventricular function is improved and sustained; 3) quality of life is improved; and 4) excellent long-term survival is attained. Bypass surgery should be applied aggressively to patients with severe, proximal coronary artery disease and severely depressed LV function. Coronary revascularization restores function to hibernating myocardial segments and represents a valuable alternative to heart transplantation in the patient with advanced ischemic cardiomyopathy.

**Does c-kit Blockade Induce Apoptosis in Mouse Ovaries in Vivo?** Alison E. Zimon, Hugh S. Taylor, and Ervin E. Jones. Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

This study aimed to assess the hypothesis that the growth factor receptor c-kit regulates apoptosis in the prepubertal mouse ovary. Employing an *in vivo* mouse model, c-kit translation was locally blocked by intra-ovarian bursal injection of oligonucleotides antisense to c-kit in 20-day-old gonadotropin-primed female mice. Effects of the antisense treatment were assessed using DNA fragmentation analysis and the terminyl deoxynucleotidyl transferase-mediated DUTP nick end-labeling (TUNEL) technique and compared to effects of missense oligonucleotide treatment. DNA laddering, a hallmark of apoptosis, was induced in ovaries by the c-kit antisense treatment six hours post-treatment and was significant compared to minimal laddering induced six hours following missense treatment. No significant differences between treatment groups were observed 2, 4, 8, 18, and 24 hours following treatment. To determine whether these results could be confirmed by an alternative method of apoptosis detection, the *in situ* TUNEL technique was applied, however a functional assay could not be established. The results of this study indicate that apoptosis may be induced in prepubertal mouse ovaries six hours following local blockade of c-kit using antisense oligonucleotides, although confirmatory studies are required.