Thesis Abstracts 1996
Yale University School of Medicine

Potential Direct Medical Cost Savings Secondary to Improved Physical Fitness Within the Late Middle-Aged and Elderly Populations. Murad Alam (Sponsored by Ethan R. Nadel). Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

This paper quantifies the potential medical cost savings that may be achievable if a significant proportion of the middle-aged and elderly population in the U.S. came to enjoy a heightened level of fitness.

The initial part examines the literature regarding specific diseases and health domains. The best available data on the impact of physical fitness on the disease states are summarized, and the attendant risk reductions, if any, are explicitly noted. In the final part of the paper, the most rigorously derived available estimates of total health care expenditures in the areas discussed are presented. Based on the previously expressed risk reductions, dollar amounts for potential cost savings in each category are derived. Thus it is demonstrated that improved physical fitness would not only reduce suffering and extend functional life-span in older Americans, but also generate tangible and substantial cost savings for the medical care delivery system as a whole.

The dollar amount of potential savings is found to be significant. Building on the savings estimates derived for each category of disease examined, a $90 billion (10 percent) total reduction in health-care expenditures can be postulated. Decreases in costs due to cardiovascular disease, institutionalization/nursing home admission, occupational and other injuries, and osteoporosis contribute the largest shares. A variety of other disease processes, notably stress urinary incontinence, diabetes, certain location-specific cancers, upper respiratory tract infection, and depression, account for an additional $20 billion in unincurred costs.

In sum, the effects of physical fitness on health and medical costs are unequivocal. Especially in the elderly, regular physical activity directly contributes to the postponement or avoidance of significant morbidity, thus reducing utilization of medical services.

Ethics or Etiquette: The History and Epidemiology of Professional Courtesy in Medicine. Jeffrey I. Algazy (Sponsored by Donna Diers). Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

This study is an investigation of the historical origins, epidemiology, and future of professional courtesy in medicine. Professional courtesy is defined as the waiving of all or part of a physician’s fee incurred when a colleague seeks medical care. Professional courtesy, as envisioned by physician Thomas Percival in 1803, was originally intended to prevent practitioners from treating themselves or their families. An historical review of the practice was conducted through library and archive research. National surveys on the topic of professional courtesy were reviewed from Medical Economics, the Journal of the American Medical Association, and the New England Journal of Medicine. Research concerning physician self-treatment and treatment of family members was studied to investigate these practices within the medical profession. The historical development of organized guidelines (codes of ethics) on the subject of professional courtesy and the treatment
of physician-families was traced from Hammurabi to contemporary medical school oaths. Ethical codes, since their origins in medicine, have never solely dealt with ethics, but have served more as guides to professional conduct and professional etiquette. This study presented evidence that the number of physicians providing professional courtesy has not declined significantly over the past forty years. Over 90 percent of practitioners today still provide some form of professional courtesy to their physician-patients. Even so, physicians provide inadequate and inappropriate care for themselves and their families. Professional courtesy is no longer the means of preventing such misguided self-treatment. Questions are now raised about the ethics of forgiveness of copayments as professional courtesy (the most common form of the practice). Medico-legal purists may consider this form of courtesy as fraud or an abuse of "antikickback" statutes. These types of significant changes in medicine will result in the future disappearance of the professional courtesy tradition.

A Review of Primary Care for Hypertension in a South African Day-Hospital. Chidinma N. Alozie (Sponsored by Jody Sindelar). Division of Chronic Diseases of Lifestyle, Medical Research Council, Cape Town, South Africa.

Hypertension is a major cause of morbidity and mortality in South Africa; the crude prevalence rate is estimated at 18 to 25 percent. The poor rates of diagnosis, treatment, and control of high blood pressure indicate that there is a need for the revision of hypertension control strategies in the country. There are two public health strategies documented to reduce the risk of hypertension related disease: the clinic-based strategy and population-based strategy. Given the limited funds for health care in South Africa, recommendation of either strategy should be based on issues of costs and effectiveness. Primary care for hypertension at the Mitchell’s Plain Day-Hospital was evaluated to identify factors influencing the poor rates of blood pressure control in the population. Cost-effective interventions for the management of hypertension from the literature were evaluated for their applicability to the study population. Poor compliance and lack of knowledge are major contributors to the low rates of blood pressure control in the Mitchell’s Plain population. Due to inadequate data, no conclusions can be made about the cost-effectiveness ratio of the population based strategy in comparison to that of the clinic-based strategy. The population-based strategy does increase the awareness of disease and thus can be used as an adjunct to other strategies. The clinic-based strategy was found to be cost-effective. However, factors such as compliance and society’s willingness to pay for a unit of health benefit vary significantly between societies. Thus the applicability of data in the literature to the Mitchell’s Plain population is questionable. To identify interventions that are cost-effective for the South African setting, studies evaluating costs and health benefits need to be conducted within the country.

Crosslinking CD28 Reverses Negative Signalling in a Th2 Clone. Oliver M.L. Bacon and Charles A. Janeway, Jr. Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut.

While the engagement of the CD4 coreceptor has been shown to augment helper T-cell activation 30 - 300 fold, it can also, under different circumstances, block activation, an in vitro phenomenon known as negative signalling. This occurs when T-cell proliferation, induced by anti-TCR mAb and IL-1, is blocked by the addition of IgM anti-CD4 mAb. This phenomenon is thought to mimic the in vivo situation in which peripheral T-cells
refrain from activation upon encountering cells expressing MHC class II without antigen specifically recognized by the TCR. In a series of proliferation assays, we show that negative signalling in D10, a murine Th2 clone, takes place when cells are stimulated with an αTCR mAb that does not force the association of CD4 with the TCR, and when the inhibiting αCD4 antibody has prolonged contact with the cells. We also find that negative signalling can be overcome when cells are stimulated with increasing concentrations of an αTCR mAb that does force the association of CD4 with the TCR, and when CD28 on the surface of the T-cell is crosslinked. Together, these findings support previous suggestions that negative signalling may represent an additional function for the CD4 molecule, namely to hold Th2 activation in check as the cell sorts through APCs in the lymph node, and that negative signalling is inhibited during proper Th2 activation by primed APCs bearing antigen specific for the TCR. In a separate, preliminary experiment, we use semi-quantitative RT-PCR to show that inhibiting D10 proliferation with multivalent αCD4 mAb decreases IL-4 message strength, suggesting that decreased lymphokine stability may have some role in negative signalling.

Pilot Study to Evaluate the Impact of an Educational Video about Melanoma on Knowledge and Behavior. Sepideh Bagheri, Jean Bologna, and Marianne Berwick. Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut.

Background: Given the growing worldwide incidence and mortality of melanoma, and a lack of cure at more advanced stages of the disease, much effort has focused on educational and screening interventions aimed at diagnosing lesions at earlier, potentially more curable stages. Skin self-examination (SSE) has received increasing attention as a means of complementing public screenings; however, to date, there have been no trials specifically aimed at promoting SSEs among targeted populations.

Objective: In this pilot study, an educational video on melanoma was prepared to not only raise subjects' awareness about melanoma epidemiology, but also prompt regular SSEs (including the use of a "buddy").

Methods: Of the 94 participants recruited, 86 completed the study: 33 melanoma, 33 atypical nevus and 21 control subjects. Each subject received a total skin examination by a physician and viewed the ten-minute video, in addition to completing pre- and a post-intervention questionnaires (on knowledge and SSE practices).

Results: Of note, the melanoma and nevus groups had the highest knowledge scores, while the controls had the greatest improvement. The controls were as knowledgeable of melanoma risk factors (like red hair and blue eyes) as the melanoma and nevus subjects, but less knowledgeable about melanoma signs and symptoms. One of the greatest post-intervention changes related to awareness of such methods as the "buddy" system to examine difficult-to-see areas. Other significant predictors of knowledge increase included the hair color, the objective (physician-determined) and subjective (self-reported) nevus counts and the presence of atypical nevi. Regarding SSE practices, the melanoma and nevus subjects were significantly more likely to perform SSEs (at least once per year) as compared to the controls. However, there were no significant predictors of either "optimal" SSEs (defined as once per month to once every four months) or improved SSEs.

Conclusion: Based on our results, a video on melanoma appears to be an excellent educational medium, as observed by the significant improvements in knowledge among the controls. However, more intensive and continuous interventions may be needed in order to affect behavioral change.
The Effect of Hypoxia on the Development of Callosal Connectivity. Alison Begleiter (Sponsored by Michael L. Schwartz). Department of Neurobiology, Yale University School of Medicine, New Haven, Connecticut.

Hypoxia is associated with intraventricular hemorrhage, the most common central nervous system abnormality in preterm infants and the major contributor to the neurodevelopmental impairments found in these infants. The purpose of this research study was to examine the effects of hypoxia on the organization of callosal connections. The development of callosal neurons occurs in two distinct phases: first, a stage of overproduction in which fibers sprout out uniformly; second, a pruning back stage during which specific fibers are strengthened while others are eliminated in an activity-dependent manner. It was hypothesized that since hypoxia causes a generalized activation of neuronal pathways, the callosal architecture in rats reared in hypoxic environments would more closely resemble the exuberant immature pattern than the ordered mature pattern. To test this hypothesis, the retrograde fluorescent tracer Fast Blue was unilaterally injected into the visual cortex of rats reared in either normoxic or hypoxic environments from postnatal day 3 (P3) to P33. After a one-week period to allow for transport of the tracer, the rats were perfused and brains were frozen-sectioned. The pattern of callosal projection neurons in the non-injected hemispheres was examined under fluorescence. These data reveal that 1) Areas 17 and 18a, while primarily acallosal in normoxic rats, have a substantial distribution of callosal neurons in hypoxic rats; 2) The border zones between areas 17 and 18 as well as areas 17 and 18a have a wider distribution of callosal neurons in hypoxic versus normoxic rats; and 3) The lack of callosal neurons in areas 17 and 18a of normoxic rats reflects a loss of neurons predominantly from supragranular layers during normal development. Thus, the pattern of callosal connections in the brains of the hypoxic rats was significantly more uniform and exuberant than the discretely ordered array observed in the normoxic rats. These results suggest that conditions of hypoxia prevent the activity-dependent elimination of neurons during the second stage of callosal development.

Effect of Transforming Growth Factor-β1, Fibronectin and Plasminogen Activator Inhibitor on Migration of Bovine Aortic Endothelial Cells. Christine Brozowski (Sponsored by Joseph A. Madri). Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

Restenosis remains the largest complication of endarterectomy and balloon catheter angioplasty. Vascular trauma caused by procedures such as these result in deundation injury which may lead to hyperplasia and the development of a neointima. Neointimal formation depends on smooth muscle cell proliferation, migration and matrix synthesis, and the presence of endothelium. Growth factors have been shown to have an influence on smooth muscle cells and endothelial cells. The target of this investigation was the effect of transforming growth factor (TGF-β1), fibronectin (Fn) and plasminogen activator inhibitor (PAI-2) on migration of bovine aortic endothelial cells. TGF-β1 decreased migration by 28 percent ± 3 percent. Fibronectin decreased migration by 19 percent ± 4 percent. Both values were significant (p < .05) using student's t-test. PAI-2 at 10 µg/ml did not significantly decrease migration (6 percent ± 4 percent). Later studies in our laboratory using higher levels of PAI–2 of 50 µg/ml resulted in a decreased migration of 10 percent ± 3 percent, which was statistically significant. TGF-β1 has been shown to be present in significant quantities at sites of vascular injury in vivo. TGF-β1 decreases the rate of endothelial cell migration and increases the rate of smooth muscle cell migration. Therefore, the presence of TGF-β1 at the site of a vascular lesion increases the likelihood
of restenosis. Since fibronectin can partially mimic the effects of TGF-β1 on BAEC migration, this suggests that the TGF-β1 effect on migration is mediated by modulation of the extracellular matrix. The effect of TGF-β1 on endothelial cell migration may also be due to increased expression of PAI or decreased uPA expression, as PAI-2 decreases BAEC migration.

**Osteocalcin Biosynthesis in the Hypophosphatemic Mouse Osteoblast.** Duane A. Bryan (Sponsored by Caren Grundberg). Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

X-linked Hypophosphatemic Rickets (XLH) is a disease of defective bone mineralization resulting in rickets and osteomalacia. The primary defect is abnormal renal reabsorption of phosphate resulting in hypophosphatemia. Until recently, ambient hypophosphatemia had been thought to be the root cause of the bony abnormalities observed. However, recent data suggest that a primary defect may also exist in the osteoblast. The Hyp mouse is the murine homologue of XLH and serves as an ideal model to study the role that ambient hypophosphatemia vs. a primary cellular defect may play. Osteocalcin is a specific product of the osteoblast and serves as an index of osteoblastic activity. Previous data showing abnormal regulation of osteocalcin synthesis in the Hyp mouse by 1,25(OH)_{2}D_{3} may suggest an intrinsic defect in the Hyp osteoblast. It was our purpose to further examine these findings *in vitro* in order to eliminate confounding variables, which may have been responsible for this difference *in vivo*.

We isolated normal and Hyp osteoblasts in culture and studied the effect of 1,25(OH)_{2}D_{3}, a known trophic factor for osteocalcin synthesis in normal cells. mRNA was isolated from cell layers for Northern blot analysis. In other experiments, we assessed the effect of ambient phosphate concentration on osteocalcin production in normal cells by growing osteoblasts in low (0.5 mM) normal (3.5 mM) and high (5.0 mM) phosphate concentrations. Finally, we attempted to assess the role of mineralizing agents on osteocalcin production by supplementation of β-glycerol phosphate.

We found that indeed Hyp osteoblasts showed a paradoxical response to 1,25(OH)_{2}D_{3} stimulation. While normal osteoblasts showed almost a two-fold increase in osteocalcin production with 1,25(OH)_{2}D_{3}, Hyp osteoblasts seemed to be inhibited (p < .001). We also showed this difference to exist at the transcriptional level. We were not able to show significant difference in normal osteoblasts by altering ambient phosphate levels. Also, mineralizing agents increased proportion of osteocalcin being expressed in matrix as opposed to media and at earlier timepoints.

**The Presence of Myofibroblasts In Contracting Ligaments.** Nelson Charlie, Michael E. Wiggins, and Joseph A. Madri (Sponsored by Peter Jokl). Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study is to identify the presence of myofibroblasts in contracting ligaments at three, six and nine weeks after a traumatic injury to the medial collateral ligament. It has been proposed that the mechanism of ligament contracture involves a contractile fibroblast known as the myofibroblast. Using alpha-smooth muscle actin immunohistochemical stain, previous studies have looked for myofibroblasts at time intervals no greater than four weeks in contracted ligaments due to immobilization. In this study, 11 skeletally mature female New Zealand white rabbits, age six to eight months were subjected to a complete traumatic tear of the midsubstance of the ligament. Both
Comprehensive Electrophysiologic Evaluation of Patients with Congestive Heart Failure. Allan Chen, Tarik R. Ramahi, and Craig A. McPherson. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Patients with congestive heart failure (CHF) who evidence nonsustained ventricular tachycardia (NSVT) are at increased risk for sudden cardiac death. Sixty-five patients with CHF due to either coronary artery disease (26 patients) or idiopathic dilated cardiomyopathy (39 patients) and no spontaneous sustained ventricular tachycardia (VT) underwent electrophysiologic (EP) testing for evaluation of NSVT, frequent ventricular ectopy, or arrhythmia risk while awaiting heart transplantation. All patients had a left ventricular ejection fraction (LVEF): less than or equal to 55 percent, and the mean LVEF was 20 ± 8 percent. Six EP parameters (sinus cycle length, His-Purkinje interval, connected sinus node recovery time, sinoatrial conduction time, Wenckebach cycle length and ventricular effective refractory period) were compared between surviving patients and deceased patients. No EP parameter was found to correlate with outcome. Programmed electrical stimulation (PES) was performed at two right ventricular sites at two drive lengths using up to three extrastimuli. Sustained monomorphic VT was induced in 14 percent of study subjects. Twenty-three percent of patients were considered "inducible" by the attending electrophysiologist and at high risk for recurrent arrhythmia. Ventricular responses of more than 10 beats and more than three beats were inducible in 32 percent and 63 percent of subjects, respectively. After a mean follow-up period of 21.7 ± 16.1 months, 60 percent of studied patients were alive, 22 percent were deceased and 18 percent had been transplanted. Actuarial survival curves were created, and one and two-year mortality rates for the study population were 11 percent and 25 percent, respectively. The results of PES were not predictive of outcome in patients with either coronary artery disease or idiopathic dilated cardiomyopathy. Comprehensive EP testing offers no prognostic information in patients with CHF and no history of sustained VT.
Assisted Reproductive Technologies: Legal Issues in Procreation. Roger J. Chin (Sponsored by Angela R. Holder). Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

This thesis examines the legal constraints on the denial of assisted reproductive services. The technical and policy background surrounding the provision of assisted reproductive technologies is surveyed. Those unfortunately saddled with infertility face barriers to access on a number of levels: from health care providers, third-party payers, and the state. While efforts to regulate natural procreation have been discredited by the failure of the eugenics movement, access to assisted reproductive technologies is often denied based on judgments of parental fitness.

The constitutional protection of procreative rights and statutory guarantees of access to medical care must be weighed against state interests in regulating these technologies. Because of the recent advent of these reproductive possibilities, legal precedents and regulations have failed to contemplate the new conflicts that arise. However, an analysis of the values underlying the legal doctrines and the jurisprudence in analogous situations reveals the scope of the right to procreation. While new social and gestational combinations in parenthood may be beyond the contemplation of due process protections, the utilization of the new reproductive technologies in procreation is as much implicit in the concept of ordered liberty as judicial precedent has recognized traditional coital reproduction to be. The state interest in the fetus or the future family outcome is not sufficiently compelling to justify the denial of these reproductive services.

The Influence of Joint Position on Fracture Type and Severity in Proximal Interphalangeal Joint Injury. John Y. Choi and Joseph F. Slade, III. Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

Proximal interphalangeal (PIP) joint position determines which joint ligaments are taut and provide stability. The purpose of our research was to develop a cadaveric model of PIP joint injury to investigate the role of joint position. We hypothesized that joint position has no influence on bony or soft tissue injury. Seventy digits in $-30^\circ$ to $40^\circ$ of joint flexion were placed in an impaction jig. A drop-weight drove an impactor into the digit, delivering an axial load. Specimens were examined for soft tissue and bony injury. ANOVA and chi-squared were used for analysis. Dorsal and volar fracture-dislocations, pilon fractures and incidental proximal phalanx fractures resulted. Dorsal fracture-dislocations occurred in 78 percent of extended digits and declined to 35 percent with flexion. Pilon fractures increased from 22 percent in extension to 35 percent in flexion. No volar fracture-dislocations occurred in extension but appeared with flexion. Proper collateral ligament (PCL) injury increased from extension to flexion at four percent to 13 percent while volar plate (VP) injury decreased from 100 percent to 56 percent. Dorsal fracture-dislocations had the greatest pre-impact hyperextension at $31^\circ$. All these results were significant. Our model generated fracture types seen clinically and supports axial loading as a mechanism of PIP joint injury. The only variable throughout the arc of motion in the PIP joint is differing tensions of the PCL and VP; in extension the VP is taut and stabilizes the joint, while in flexion the PCL is taut. We speculate that PCL laxity in extension allows for dorsal translation of the middle phalanx with axial loading.Digits with a lax VP and large hyperextension are more inclined to sustain dorsal fracture-dislocations. With flexion, the PCL is drawn taut and allows for little migration of the middle phalanx with axial loading. Energy now can only be dissipated in the form of a pilon fracture.
The Effect of Supplementation with Retinol and β-Carotene on Pulmonary Macrophage and Serum Levels of Retinol, Carotenoids and Alpha-Tocopherol. Sarah L. Clever, Ariette van Bennekum, William S. Blaner, and Carrie A. Redlich. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Much interest has been generated in the past decade by epidemiologic, in vitro and in vivo data suggesting that β-carotene and vitamin A (retinol) may play a role in resistance to cancer. Two recent large-scale β-carotene supplementation trials, however, have resulted in paradoxical increases in rates of lung cancer among high-risk individuals. There are little data on the effects of supplementation with vitamin A and β-carotene on lung parenchymal levels of these and other nutrients. The aim of this study is to determine these effects.

Twenty-one men with occupational histories of asbestos exposure were randomized to receive placebo or 25,000 IU retinol and 30 mg β-carotene per day for six months. Baseline and post-intervention serum and bronchoalveolar lavage (BAL) cell levels of these nutrients, other carotenoids and α-tocopherol were measured using HPLC methodology. Serum levels of β-carotene increased from 157 ± 103 to 1969 ± 1351 ng/ml (p < .001), α-carotene levels increased from 18.9 ± 7.1 to 64.8 ± 38.4 ng/m (p < .001), and lycopene levels increased from 296 ± 214 to 320 ± 180 (p = .04). Serum levels of retinol, α-tocopherol, β-cryptoxanthin, and zeaxanthin were not significantly changed. BAL cell levels of β-carotene increased from 2.4 ± 2.6 to 24.3 ± 28.5 ng/106 cells (p = .03). BAL cell levels of the other nutrients, including retinol, were not significantly altered, though the increase in levels of α-carotene approached statistical significance.

It can be concluded from these results that six months of supplementation with β-carotene and retinol increases serum, BAL cell and possibly lung tissue levels of β-carotene. There was no significant change in serum or BAL cell levels of retinol in spite of supplementation. The results of the intervention trials may be explained by the effect of alteration of tissue levels of micronutrients on as yet unappreciated micronutrients or biochemical pathways.

The Epidemiology of Phalangeal and Proximal Interphalangeal Joint Injuries of the Hand. Simon M. Cornelissen (Sponsored by Joseph Slade). Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

This study was performed to elucidate patterns of hand injury in the population served by Yale-New Haven Hospital and compare current trends with those seen earlier, focusing on injuries to the proximal interphalangeal joint. A retrospective chart review was used. Computer searches of billing records identified patients diagnosed with hand injuries between January 1, 1992, and January 30, 1996. These searches included both inpatients and outpatients. Three thousand seven hundred and fifty-three patients were identified. Four hundred and eighty charts of patients with phalangeal injuries in 1995 and 1994 were reviewed, and the age, gender, etiology and date of injury was recorded. A predominance of young males was found in all types of hand injuries, but this trend was most evident when only fractures were considered. Open injuries were far more likely to result in inpatient treatment than closed injuries. Ball sports caused the greatest number of phalangeal injuries (31 percent), followed by falls (16 percent), machines at work or at home (11 percent), and non-ball sports (9 percent). Football, baseball, snowblowers and firecrackers caused injuries in a seasonally-dependent distribution, whereas basketball did not show seasonal variation. Injuries to the proximal interphalangeal joint had a tendency to be more common in the little finger, and less common in the thumb. This was in contrast
with the even distribution among fingers that is apparent when all phalangeal injuries are considered. Hand injuries are common and important. Among them, proximal interphalangeal joint injuries show a different pattern of occurrence. Preventative plans can be made with the knowledge of the epidemiology of these injuries.

**Magnetic Resonance Imaging of the Ovary: Can it Predict Low Response to Empiric Superovulation?** Rebecca J. Crichton, Robert N. Troiano and David L. Keefe. Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this pilot study was to evaluate whether or not there are morphologic differences in ovarian volume, follicular size and number, as seen on Magnetic Resonance Imaging (MRI), between the ovaries of women with low response to ovulation induction and younger women with presumably normal ovarian function. Five women with an average age of 42 and a documented low response to superovulation with human menopausal gonadotropins, indicated by a peak estradiol less than 500 pg/ml, underwent MRI on the third day of their cycles. Their images were compared to the day three MRI's of five controls with an average age of 25. There was no significant difference between the ovarian volumes in the two groups. There were, however, significant differences in follicle numbers. For follicles greater than or equal to 5 mm in diameter, there were 3.2 ± 2.7 in the women with low response and 23.0 ± 3.7 in the controls (p < .0005). For follicles less than 5 mm in diameter, there were 20.2 ± 7.0 in the low-responding women and 40.8 ± 12.1 in the control group (p < .01). In terms of total follicle numbers visualized on MRI, there were 23.4 ± 7.0 in the women with low response and 63.8 ± 14.4 in the controls (p < .001). These results indicate that MRI is indeed capable of measuring differences between reproductive subgroups. Further studies are necessary to evaluate the full potential of MRI to prospectively assess ovarian reserve.

**Ouabain Potentiates Kainate Neurotoxicity: A New Rat Model of Human Temporal Lobe Epilepsy.** Amos O. Dare (Sponsored by Nihal de Lanerolle). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Neurodegeneration (cell death) is a feature of many neurological diseases including human temporal lobe epilepsy (TLE). Recently, it has been suggested that excitotoxicity, a process by which neurons are killed by prolonged or excessive exposure to glutamate or its analogs, may contribute to neuronal injury observed in many diseases characterized by neurodegeneration. Thus, in a model of TLE, the potent glutamate analog kainate has been shown to induce seizures and subsequent cell death in rat hippocampus. Although the pathology produced with this model resembles that observed in human TLE, it does not completely replicate it. Accumulating evidence now suggest that activity of the Na⁺/K⁺—ATPase enzyme (Na⁺/K⁺ pump), the ion pump responsible for neuronal ionic homeostasis, may be compromised or insufficient in many neurodegenerative diseases, particularly in TLE. In this thesis project, the hypothesis that a reduction of Na⁺/K⁺ pump capacity will reduce neuronal survival of excitotoxicity and produce a pathology in young adult rats similar to that observed in human TLE was investigated.

To test this hypothesis, brain Na⁺/K⁺ pump capacity was partially inhibited in young adult rats treated with subtoxic dose of kainate. Seizure activity in these animals was verified by electroencephalography (EEG) and damage to the hippocampus was documented by a silver staining method for dead/dying neurons. Control animals were treated with
either kainate or the Na*/K* pump inhibitor, ouabain, alone. In further studies, the chronic pathological and behavioral consequences of these treatments were determined. The following features of human TLE were studied in these animals: 1) a pattern of cell death in the hippocampus described as Ammon's horn sclerosis; 2) spontaneous, recurrent seizures; and 3) evidence of hippocampal remodeling.

Results of these experiments revealed that partial inhibition of the Na*/K* pump in young adult rats with ouabain was not itself neurotoxic. This treatment, however, markedly potentiated ordinarily subtoxic doses of the glutamate analog, kainate. Thus, rats treated with intraperitoneal (i.p.) injections of kainate (5 mg/kg; i.p.) followed by intraventricular (i.c.v) ouabain (3 nmoles) experienced behavioral and EEG recorded seizures. Subsequent silver staining of the brain of these animals showed hippocampal damage. In contrast, ouabain alone or kainate alone did not produce seizures or hippocampal damage (p < .001; chi-square analysis). These results were replicated with intraperitoneal injections of both kainate (7 mg/kg; i.p.) and ouabain (1 mg/kg; i.p.). Furthermore, Na*/K* pump impairment was found to be associated with kainate treatment in producing seizure activity and subsequent neuronal death. Thus, when ouabain (3 nmoles; i.c.v) was administered (90 min instead of 30 min) after kainate (5 mg/kg; i.p.), seizure onset was also delayed and seizure duration was reduced. Finally, treatment with the combination of ouabain and kainate produced features commonly observed in human TLE: 1) acute limbic type seizures; 2) a pattern of cell death in the hippocampus replicating that observed in the human disease; 3) a chronic epileptic state characterized by spontaneous, recurrent seizures; 4) evidence of plasticity and hippocampal remodeling.

The results of this study demonstrated a critical role for the Na*/K* pump in neuronal survival of excitatory stimuli. The hypothesis that reduction of Na*/K* pump capacity decreases neuronal threshold for hyperexcitability and injury was supported, producing a seizure syndrome and pathology in adult rats closely replicating human TLE.

**Impact of Uterine and Postnatal Growth Achievement on the Development of Neonatal Necrotizing Enterocolitis.** Michael J. Davidson, Richard A. Ehrenkranz, and Robert J. Touloukian. Department of Surgery and Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonate but remains a disease of unclear etiology. While preterm, low-birth-weight infants have the highest risk for this disease, the relationships of intrauterine and neonatal growth to NEC have not been fully defined. The aim of this retrospective study was to determine the effects of pre- and postnatal growth on the subsequent development of NEC.

Data were collected from the medical records of all patients with surgically confirmed NEC treated between January 1987 and September 1995 at Yale-New Haven Hospital (n = 69). Infants were classified as small for gestational age (SGA) or appropriate for gestational age (AGA) using the Lubchenco Intrauterine Growth Data with a tenth percentile cut-off. Postnatal growth achievement (PGA) was defined as the difference between the infant's weight at the time of NEC and the birth weight (BW), expressed as a percentage of the BW. PGA was compared to a NEC-free control group (n = 69) individually matched by gestational age, BW ± 10 percent, and date of birth. Results were analyzed using Chi-square for BW data and paired Student's t-test for PGA data.

Overall, 12/69 (17 percent) of NEC infants were SGA, compared to 10 percent predicted (p = .04). The prevalence of SGA rose with increasing age at NEC onset, affecting 13 percent of infants developing NEC at 0 to 14 days (p = NS), 18 percent at 15 to 28 days
Correlation Between Number of Dengue Virus-Infected Peripheral Blood Mononuclear Cells and Clinical Severity of Disease. Javier J. Davila and David W. Vaughn (Sponsored by Rebecca Rico-Hesse). Virology Department, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

The purpose of this study was to determine whether a correlation exists between the number of dengue virus-infected peripheral blood mononuclear cells (PBMCs) and clinical severity of disease. This would provide a method of identifying patients likely to develop severe symptoms early in the course of illness.

Blood samples were collected before defervescence (T > 38°C) from patients presenting at Bangkok Children's Hospital in June to July 1993 with suspected dengue. PBMCs were isolated and fixed on ten slides for each patient. Dengue-infected cells were visualized using murine anti-dengue monoclonal antibodies followed by biotinylated anti-mouse IgG and streptavidin-alkaline phosphatase. Positive controls were dengue-infected Vero cells while both Vero cells and normal human PBMCs were used as negative controls. Dengue antigen-positive cells in all ten slides were counted and tabulated.

There was an observable trend for those cases with fewer infected cells to be milder than those with higher infection rates. Dengue fever cases showed no more than three infected cells while none of the patients who developed dengue hemorrhagic fever grade III showed less than 7. The correlation, however, was not statistically significant (.4399, n = 15). The immunohistochemical staining method used was shown to be of value as a diagnostic tool since it agreed well with serological results.

Although a significant correlation was not established with 15 patients, a larger study may be warranted. The ability to identify potentially lethal dengue cases early on would allow a prioritization of care with increased monitoring and support for these individuals.

The Topological Distribution of Olfactory Receptor Neuron Axons in the Olfactory Bulb Glomeruli of the Rat: A Confocal Microscopic Study with Dil Staining. Jeffrey M. Dembner and Charles A. Greer. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The olfactory system encompasses three anatomical sites. Initial detection begins in the posterior aspect of the nasal cavity where specialized olfactory receptor neurons transduce odorants into neural signals. These signals are then transmitted to the central nervous system where they undergo elaborate processing within spherical shaped regions of neuropil in the first relay station of the olfactory pathway, the olfactory bulb glomeruli. The resulting bulbar signal is then propagated to numerous cortical structures. Although the precise code employed by the olfactory system to discriminate between individual odorants remains to be elucidated, the existence of an odortopic map is supported by converging
lines of evidence. The singularly unique pattern of activity across the glomerular layer of the olfactory bulb produced by a particular odorant appears to encode its identity. The activity of any single glomerulus thus represents the fundamental unit of this code. Furthering an understanding of olfactory processing, therefore, requires a detailed analysis of the anatomy of those processes contributing to the complex neuropil of the olfactory bulb glomerulus, especially that of the primary afferent projections from the neuroepithelium. It has been previously demonstrated in Golgi-impregnated specimen that single olfactory receptor neuron axons occupy only a limited region of the glomerular neuropil (Hálasz and Greer, 1993). In the present study, we extend these findings by performing a confocal examination of the distribution of Dil stained axons and fascicles as they approach, penetrate and arborize within glomeruli. Olfactory bulbs from two week old Sprague-Dawley rats were immersion fixed in four percent paraformaldehyde, implanted with 2-3 Dil crystals in the olfactory nerve layer, incubated at room temperature and in the dark for an average of 3.5 weeks and cut transversely at 100 μm with a vibratome. Thin optical images, 0.5 to 1 μm, were then captured in series with a BioRad 600 confocal laser and reconstructed in two and three dimensions for analysis. Single axons were easily resolved and exhibited both en passant varicosities and terminal boutons among intraglomerular axonal collaterals, which ranged from 2-6. Consistent with Golgi-impregnated samples, individual axons appeared to arborize within only a small proportion of the glomerulus. Most significantly, fascicles of primary afferent axons were demonstrated to distribute within spatially restricted domains, rather than ramifying homogeneously throughout the glomerulus. The terminal arbor of olfactory receptor neuron axons from the same fascicle appear to colocalize within a limited region of the glomerular neuropil, mostly segregated from the terminal arbors of those axons from a different fascicle. In addition, the terminal arbors of primary afferent fibers appear to be concentrated in the peripheral shell of the glomerulus with few penetrating into the glomerular core. These data thus support a sub-compartmental organization of the olfactory bulb glomerulus. Glomerular sub-compartment may represent the segregation of parallel afferent pathways from the neuroepithelium to the olfactory bulb, or alternatively, could be involved in the individualized processing of differential odorant information within a single glomerulus.

A Seroepidemiological Survey of Arboviruses on the Ilhas de Abaetetubata, Para, Brazil. Steven E. Dresang, Amelia P.A. Travassos da Rosa, Pedro F.C. Vasconcelos, Nicolas Dégallier and Robert E. Shope. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut; Instituto Evandro Chagas, Belém, Brazil; and ORSTOM, Montpelier, France.

Recent history has demonstrated the need to find safe, productive, sustainable forms of agriculture in ecologically threatened areas such as the Amazon. The caboclos of the Brazilian Ilhas de Abaetetubata currently practice a combination of hunting, fishing, forest extraction and small-scale farming in the tide-influenced region of the Amazon known as varzea forest. A serological survey of arboviruses was conducted with the purpose of evaluating virus exposure patterns among the caboclos. Human blood sera samples from caboclos were tested for hemagglutination inhibition antibodies against antigens from 18 different arboviruses. Forty-three of the 318 people tested had measurable antibodies. The prevalence of specific antibodies against the majority of viruses was less than one percent. Specific antibody titers were defined as four times the titer of other viruses in the same antigenic group. Yellow fever antibody prevalence was seven percent, which is low considering the availability of a vaccine. A trend (χ² = 3.28; p < .10) for increased prevalence of antibodies
among people with high levels of occupational forest contact suggests that infection is occurring primarily within the forests. Viruses with bird and rodent hosts were present, but none of the viruses with primates as their primary hosts (except possibly yellow fever) showed clear evidence of being present in the study area. Based on the antibodies present, Culex sp., Psorophora ferox and Aedes serratus appear to be the most important vectors in the study area. The caboclos' agriculture is a model of varzea forest management that currently does not cause frequent exposure to arboviruses. The low level of immunity, however, leaves the caboclos highly susceptible if conditions are modified in a way that increases arbovirus transmission. As part of a larger study, the results will be used to guide further studies of the ecology of the viruses in an attempt to promote safe, productive and sustainable agriculture.

Processing Strategies for Functional Magnetic Resonance Imaging of the Visual System in Occipital Lobe Epilepsy. Richard W. Epstein, Adam Anderson and Edward J. Novotny, Jr. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

This study investigated processing strategies to optimize the detection of functional changes in the occipital lobe of patients with occipital lobe epilepsy.

Functional magnetic resonance imaging (fMRI) studies of the visual cortex were performed on 10 patients with occipital lobe epilepsy and 14 normal controls. The visual stimuli included a baseline fixation task and an activation task using a full-field checkerboard flickering at 8 Hz. Activated voxels were detected using a split-2 Student's t-test and activation was quantitated using the percent difference. Three slices covering the occipital lobe were analyzed. Regions of interest (ROI) were defined for the entire occipital lobe (large ROI) and for the medial cortex (small ROI). The mean activation and asymmetry between the two hemispheres were determined at various thresholds. Each subject had a control region of interest defined in the frontal lobe were no activation was expected.

The mean asymmetry for patients was greater than that for controls in the two ROIs and at all thresholds. The most statistically significant difference between mean asymmetries was found using the small ROI and a threshold of 2.0 (p = .02). The mean asymmetry was 0.27 for patients and 0.15 for controls. In seven of the eight patients with a unilateral epileptogenic focus, the hemisphere with the focus had less activation. The control ROIs in the frontal lobe showed no difference.

Functional magnetic resonance imaging can be used to measure asymmetric occipital lobe activity in patients with occipital lobe epilepsy. As a group, there was a greater asymmetry in epilepsy patients than in normal subjects, but there was overlap between the populations. The epileptogenic focus was most often in the less active hemisphere. Detailed ROIs improved the sensitivity of this measure. These studies are limited by the patient's ability to comply with the visual fMRI protocol.

Analysis of Cytotoxicity and Invasiveness of Bacteria Isolated from Bottled Water. Monica Escarzaga (Sponsored by Stephen C. Edberg). Departments of Laboratory Medicine and Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The Food and Drug Administration is proposing that the Quality Standard for bottled water require the absence of coliform bacteria and is further requesting that the agency should establish quality standard regulations for other microorganisms that may
be present and pose a health risk. The following study examines the virulence of bacterial strains found in bottled water by assessing their cytotoxicity and invasiveness. Bacterial samples were collected from bottled water by filtration and plated on Blood Agar plates. Growing colonies were isolated onto additional Blood Agar plates and used for the preparation of overnight cultures. Aliquots were taken from each of the overnight cultures and used to grow the bacteria to log phase. The remainder of each of the overnight cultures, with bacteria in static phase, were used to inoculate C2bBe monolayers, a subclone of Caco-2 cells with enterocytic differentiation. To test for invasiveness, C2bBe monolayers were inoculated with both log phase and static phase bacteria. To test for cytotoxicity due to enterotoxin production, supernatant was isolated from the log phase bacterial culture and added directly to C2bBe monolayers. All monolayers were incubated for 2 hr at 37°C, with five percent CO₂. Trypan blue stain was used to determine cytotoxicity while acidine orange stain in Gey’s solution counterstained with crystal violet was used to determine invasiveness. Statistical analysis, using the test for difference between two independent counts, showed that bacteria collected from bottled water pose a low health risk, since they are not significantly cytotoxic nor invasive to gut epithelial cells. The z values for the cytotoxicity assay as compared to the control were: 1.13 for log phase, 0.96 for supernatant, and 0.21 for static phase. The z values for the invasion assay as compared to a positive control were 3.92 for log phase and 5.19 for static phase. It is, therefore, concluded that the majority of bacteria from bottled water, being neither cytotoxic nor invasive, are unlikely to cause disease after ingestion. The FDA’s proposed amendment to the Quality Standard for bottled water is, therefore, deemed as unnecessary.

**Effect of Oropharyngeal Stimulation on Fluid Intake after Exercise/Thermal Dehydration in Humans.** M.K. Figaro (Sponsored by E. R. Nadel). John B. Pierce Laboratory and Yale University School of Medicine, New Haven, Connecticut.

We examined the effect of oropharyngeal stimulation on thirst, secretion of arginine vasopressin (AVP) and fluid intake in six healthy adults following dehydration (29 ± 0.2 ml/kg water loss) induced by mild exercise in the heat (2 hr, 38°C, relative humidity < 30 percent). Subjects performed three identical dehydration protocols followed by 75 min of rehydration at 27°C consisting of 1) ad libitum drinking (CON); 2) infusion of a similar volume of water directly into the stomach via a nasogastric tube (INF) during the first 25 min and combined INF and ad libitum drinking during the remaining 40 min of rehydration; or 3) ad libitum drinking with simultaneous extraction of ingested fluid via a nasogastric tube (EXT). Plasma osmolality (Posm), AVP, sodium, fluid intake, thirst perceptions and urine output were measured throughout. On average, dehydration increased Posm 7.8 ± 0.6 mosmol/kg H₂O, thirst ratings 64 ± 8 percent, and AVP 3.1 ± 0.4 pg/ml. In CON and EXT, AVP and thirst ratings immediately decreased after drinking without any demonstrable change in Posm, while infusion of water directly into the stomach caused no significant reduction in AVP or thirst rating. Subjects replaced 20.0 ± 2.0 ml/kg or 67 percent of the water lost during dehydration over the 75 min CON rehydration period. Sham drinking (EXT) resulted in a small, yet significant, increase in the volume of water replaced averaging 31.3 ± 7.1 ml/kg EXT (p < .05) or 85 percent of the fluid losses during dehydration. These data support the hypothesis that oropharyngeal stimulation provided an important input for inhibition of thirst and AVP secretion but more importantly demonstrated the role of this reflex in limiting total fluid intake in the presence of a persistently high Posm, as seen during our sham drinking (EXT) experiments.
Gastric Carcinoid Tumors: From Cell to Survival. Christopher James Gilligan, Massimo Pignatelli, A. Brian West, Andras Sandor, Laura H. Tang, Mark Kidd and Irvin M. Modlin. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The aims of this investigation were to define the natural history of and optimal therapy for gastric carcinoid tumors, to establish the incidence of these lesions, in particular to determine whether their incidence has increased since the widespread introduction of acid inhibitory pharmacotherapy, to characterize the cell adhesion molecule (CAM) and growth factor expression of these lesions with the aim of elucidating the cellular basis of the different tumor subtypes observed clinically, and to evaluate gastrin receptor expression and function during rapid transformation of the enterochromaffin-like (ECL) in Mastomys (a rodent model for gastric carcinoids) in order to elucidate the role of hypergastrinemia in promoting ECL cell hyperplasia and neoplasia.

We have evaluated the natural history of gastric carcinoid tumors by reviewing the records of all patients treated for these lesions at Yale-New Haven Hospital from 1983 to 1993 and confirmed that lesions associated with CAG and hypergastrinemia display distinctly less aggressive behavior and thus may be justifiably treated more conservatively with surveillance, endoscopic removal or antrectomy. On the basis of the largest epidemiological investigation of these tumors conducted to date (8305 cases from the data bases of the National Cancer Institute), we report that gastric carcinoids have increased in incidence over the last 20 years. This may represent either improved diagnostic techniques, increased awareness, or a real change in incidence. Immunohistochemical evaluation of gastric carcinoid tumors reveals that they display variable positive immunoreactivity for numerous growth factors and CAMs. In addition, they display a low rate of proliferation, consistent with their relatively benign clinical behavior. The patterns of growth factor and CAM expression on these lesions may correspond with a particular tumor's metastatic potential and gastrin-dependent status. Finally, we have demonstrated a direct correlation between pharmacologically induced hypergastrinemia, ECL cell proliferation; and gastrin receptor upregulation in Mastomys, further elucidating the role of gastrin in the development of these tumors.

The Development of a Quantitative ELISA for 8-Methoxypsoralen Photoadducts: Its Use in Characterization of the Molecular Specificity of the 8G1 Antibody and Potential Use in the Prevention of Restenosis after Balloon Angioplasty. Aaron G. Grand, Louis A. Amici, Bauer A. Sumpio, Lawrence Deckelbaum, Richard L. Edelson and Francis P. Gasparro. Departments of Surgery, Internal Medicine, and Dermatology, Yale University School of Medicine, New Haven, Connecticut.

In combination with ultraviolet A or short-wavelength visible light, psoralens have been shown to have antiproliferative effects and immunomodulatory capabilities, making them invaluable in the treatment of many clinical illnesses. Another potential use for psoralens has arisen due to the increasing number of balloon angioplasty procedures performed. Balloon angioplasty may be complicated by vascular rupture, intimal tears, vasospasm and restenosis; while initially effective in 85 percent of all cases, restenosis affects up to 50 percent, necessitating repeat procedures. The pathologic processes resulting in restenosis are not entirely clear, but smooth muscle cell proliferation plays an important role. Thus, because psoralens have been demonstrated to have antiproliferative effects on smooth muscle cells, and because this effect can be achieved with exquisite localization by focal irradiation, it was proposed to examine the use of 8-methoxypsoralen
(8-MOP) in the prevention of restenosis after angioplasty. To optimize this technique, a means of measuring photomodification by 8-MOP was important, and therefore, a quantitative ELISA for 8-MOP photoadducts was developed.

High performance liquid chromatographic analysis of photomodified calf thymus DNA was performed. Quantitative ELISA measurement of known quantities of photoadducts generated a standard curve to which the unknowns were compared. Thus, unknown quantities of 8-MOP photoadducts in quantities as small as 10 femtomoles could be measured with an error usually less than 20 percent. Further characterization of the primary antibody was also performed.

The development of this assay allows for accurate determination of the degree of photomodification of DNA with 8-MOP and UVA or visible light. This assay may be useful in optimizing the treatment of vascular tissue at the time of angioplasty to prevent restenosis.

**Young Infants Who Present to the Emergency Department with Bronchiolitis and Fever: Risk of Serious Bacterial Infection and Variability in Diagnostic Evaluation.**

Karanja D. Harvey and Erica L. Liebelt. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study is to assess the risk of serious bacterial infection (SBI) in infants at or less than 90 days of age who present to the emergency department with bronchiolitis and fever, to describe the diagnostic evaluation these infants receive and to define predictors, if any, of SBI in this cohort of infants.

The study design was a cross-sectional study of all infants less than 90 days of age who presented for bronchiolitis diagnosis from November 1993 through March 1995 in the Yale-New Haven Hospital emergency department. Two hundred and eleven consecutive infants (mean age 53.5 ± 21 days) with 216 episodes of bronchiolitis were included.

One or more laboratory test (not including chest radiograph) was obtained in 53 percent of 216 episodes. Of 91 episodes among infants with fever or history of fever (temperature > 38°C), white blood cell count was obtained in 77 percent, urinalysis in 53 percent, urine culture in 60 percent, blood culture in 75 percent and cerebrospinal fluid (CSF) analysis and/or culture in 47 percent. One or more laboratory test was obtained in 79 percent. Seventy-six percent were hospitalized, and 52 percent were treated with parenteral antibiotics. In contrast, of 125 afebrile episodes, one or more laboratory test was obtained in 34 percent (p < .001), 63 percent were hospitalized (p = .001), and 10 percent were treated with parenteral antibiotics (p < .001), given comparable clinical status of febrile and afebrile infants. Of 216 episodes, none had SBI by analysis of blood, urine and CSF cultures (risk: 0 percent; 95 percent CI: 0 to 1.4 percent).

Although the risk of SBI appears low, there is marked variability in the diagnostic evaluation for SBI among young infants with bronchiolitis. Although fever or history of fever appears to be an important factor in determining whether or not laboratory tests are obtained, there was marked variation in the laboratory evaluation of both the febrile and afebrile groups.
Linkage Disequilibrium Analysis in the Primary Hemochromatosis Region, Utilizing Five Novel DNA Probes. Samantha K. Hendren (Sponsored by Jeffrey R. Gruen). Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Hemochromatosis is associated with the HLA-A3 seroantigen and several other genetic markers near the Class I MHC on chromosome 6p. Genetic linkage studies strongly suggest that hemochromatosis is due to a single-gene defect located in this region. Additional genetic markers will be necessary, however, to more precisely localize this clinically important gene. This study begins with the hypothesis that new, unique, polymorphic DNA markers from near HLA-A will be associated with the hemochromatosis gene (HFE), due to linkage disequilibrium. Furthermore, due to the strong established linkage between HLA-A and HFE, genes isolated from this region may be candidates for the HFE gene itself. The specific goals of this project included: 1) determining whether any of a collection of 11 new, unique DNA sequences from the region surrounding HLA-A identify RFLPs; 2) utilizing any polymorphic markers discovered to determine if an association exists between HFE and certain alleles of the RFLPs; 3) examining the genomic DNA of affected individuals for gross abnormalities that may be identified by hybridization with these markers; and 4) attempting to describe an ancestral haplotype in hemochromatosis patients with respect to these markers.

Hybridization of 11 small, unique DNA markers with restriction endonuclease-digested DNA from control individuals resulted in the identification of 12 RFLPs. Five of the RFLPs were examined with sufficient numbers of individuals to make a determination of association with hemochromatosis. One of the marker alleles, VLG-63 allele b, was found to be in statistically-significant association with the disease allele, with an approximate relative risk of 2.9. Another marker allele, VLG-20 allele b, was nearly statistically significant, with an approximate relative risk of 3.3. The other three RFLPs failed to show significant association with the number of individuals studied. Possible reasons for this result are extensively discussed. Gross deletions or rearrangements of patient DNA in this region were ruled out by the Southern blot data. Although formal haplotype analysis was not possible, a descriptive analysis of the data suggests a predominant ancestral haplotype with respect to VLG-10, VLG-20 and VLG-35.

Further research, utilizing disequilibrium measurements with greater numbers of individuals as well as formal haplotype analysis, is warranted on the basis of these results.

The Ming Dynasty: A Renaissance in Medical Thought. Shih–Yin Ho (Sponsored by William C. Summers). Section of History of Medicine, Yale University School of Medicine, New Haven, Connecticut.

Medicine in China has generated great interest in the West ever since trade routes and explorers linked Europe with China as early as the thirteenth century. Yet, determining the exact nature of medicine in China has proved to be a complex task, due to the diverse and nonlinear evolution of its conceptual systems. By examining medical thought within the sociopolitical and socioideological context of Chinese civilization, one gains a better understanding for how distinctly separate systems of medical ideas could coexist. This paper explores the period of the Ming Dynasty (1368-1644) and demonstrates the diversity of conceptual systems which characterized medical thought. This was an era of intellectual revival, creativit, and philosophical upheaval, which witnessed a renewal in different medical theories and practices, the result of many physician-scholars who interpreted, criticized and added to the classics of orthodox medicine. There also emerged scores of
publications, a reawakening of philosophical thinking and a redefining of political, social, and religious concepts which had been neglected in the past.

Drawing on Ming diaries, journals and casebooks from the China Academy of Traditional Chinese Medicine in Beijing, China, as well as analogies from contemporary historians, this paper defines medical thought along five different lines of conceptual approaches including: adaptations of conventional theory, etiological principles, a return to classics, medicine of "common physicians" and an impact from foreign influence and advances in medicine. The spectrum encompassed reinterpretations of the classics, conventional orthodoxy, supernatural or metaphysical concepts, demonology and pragmatic experience as well as intellectual, politico-economic, and social influences. Interestingly, as each decade passed, there emerged an increasing divergence of ideas in which no one theory gained dominance. Nonetheless, this period, which witnessed the publication of the most celebrated comprehensive Pharmacopoeia, the use of general anesthesia, and challenges to orthodox thinking, was indeed a renaissance in medical philosophy.

ATP Threshold for Loss of Epithelial Cell Polarity and Elaboration of Fodrin Cleavage Products Following in vivo Ischemic Renal Injury. C.J. Holcroft, H. Carey, S. Van Why, X. Zhu, G. Thulin, M. Kashgarian and N.J. Siegel. Departments of Pediatrics and Pathology, Yale University School of Medicine, New Haven, Connecticut.

Acute tubular necrosis is a common cause of intrinsic acute renal failure. Ischemic injury results in depolarization of the Na+/K+-ATPase from the basolateral membrane in the proximal tubule. Na+/K+-ATPase is linked to the actin cytoskeleton through two other cytoskeletal proteins: ankyrin and fodrin. With prolonged ischemia, cellular tight junctions break down and Na+/K+-ATPase disassociates from the cytoskeleton and diffuses to the apical membrane. An experimental model of acute tubular necrosis can be produced in rats after 45 min of total occlusion of the renal arteries.

For this experiment, Sprague-Dawley rats were anesthetized, the external jugular vein was cannulized, tracheotomy was performed and a pressure transducer was placed in a femoral artery. A balloon pump was placed around the aorta proximal to the renal arteries. The rat was placed in NMR, and baseline ATP levels were obtained. The balloon pump was then inflated until the pressure transducer indicated a 90 percent decrease from baseline values. The rats next underwent 45 min of graded renal ischemia, during which ATP levels were monitored. The kidneys were then harvested and the kidney cortex was homogenized in a Triton-X-100 solution. Triton-X-100 is a detergent that separates cytoskeletal-associated proteins into the insoluble fraction and unassembled, or disassociated proteins into the soluble fraction. The Triton-X-100 soluble fractions were then run on standard SDS electrophoresis and Western blots were performed with monoclonal antibodies to Na+/K+-ATPase and polygonal antibodies to fodrin and fodrin cleavage products. The quantity of proteins present in these bands were then determined by densitometry.

Initial results suggested that a threshold for Na+/K+-ATPase depolarization existed. For ATP levels under 50 percent of baseline levels, the Triton-X-100 soluble fraction of Na+/K+-ATPase appeared to be increased. Furthermore, the degree of Na+/K+-ATPase depolarization increased with decreasing ATP levels. Fodrin cleavage products appeared in the Triton-X-100 soluble lane at ATP levels at approximately 60 percent of baseline values. This suggested that fodrin cleavage may play a crucial role in the regulation of Na+/K+-ATPase disassociation from the actin cytoskeleton.

Unfortunately, variability in subsequent experiments did not fully confirm the initial results; this failure is likely due to technical difficulties.
Violence and Urban Adolescent Girls: Compounded Community Trauma, Post-Traumatic Stress Disorder, and Communal Ties. Karyn J. Horowitz (Sponsored by Stevan Weine). Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut.

This study describes the pattern of exposure to violent events, the post–traumatic stress disorder (PTSD) symptoms, and the mediating role of communal ties in a population of urban adolescent girls. Seventy-nine urban adolescent girls attending an Adolescent Medicine Clinic were assessed via a clinician-assisted self-report measure called the Adolescent Self-Report Trauma Questionnaire (ASTQ). The ASTQ gathered information on demographics, exposure to community and domestic violent events, PTSD symptoms and communal ties (three Likert variables measuring aspects of the person's involvement with communal institutions of family, school and peers). The adolescents experienced between eight and 55 different types of community and domestic violent events (mean = 28). Sixty-seven percent met symptom criteria for PTSD. T-tests showed that persons who met DSM-III-R criteria for PTSD had decreased scores for communal ties in the areas of family (p = .002), school (p = .0004), and peers (p = .05). Pearson correlations showed increased trauma exposure correlated with decreased scores for communal ties in the areas of family (r = .36, p = .0009), school (r = .42, p = .0001), and peers (r = .50, p = .0001). These urban adolescent girls experienced compounded community trauma: prolonged and repeated exposure to multiple types of community and domestic violent events, via multiple modalities of contact, over time. These findings suggest that when adolescents experience compounded community trauma, their involvement with communal institutions such as family, school and peers is a critical element in both the etiology of violence exposure and the pathogenesis of PTSD.

Assessment of Myocardial Inotropic Reserve in Patients with Idiopathic Dilated Cardiomyopathy. Elizabeth C. Hsia, Mark A. Saari, Craig McPherson and Tarik M. Ramahi. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Patients with chronic heart failure (CHF) and idiopathic dilated cardiomyopathy (IDC) have a decrease in the sensitivity of their myocardial β-adrenergic receptors proportional to their degree of left ventricular dysfunction. Assessment of inotropic reserve by dobutamine-induced augmentation of left ventricular ejection fraction (LVEF) may reflect this subsensitivity of the adrenergic system and may prove to be a useful prognostic tool. In an attempt to investigate this, inotropic reserve was assessed in 30 patients with IDC using intravenous (IV) dobutamine infusion. LVEF was measured by equilibrium radionuclide angiocardiology (ERNA) at rest and after dobutamine at 10 μg/kg/min was infused for 10 min. Hemodynamic parameters, including heart rate (HR), stroke volume (SV), systemic vascular resistance (SVR), cardiac output (CO) and mean blood pressure (MBP) were also measured both at baseline and after 10 min of dobutamine infusion. A wide variation in the change in LVEF (ALVEF) was detected, unrelated to resting LVEF (mean 26 ± 9 percent), other resting hemodynamic measurements, peak oxygen consumption (mVO₂) and other prognostic factors except right ventricular ejection fraction (RVEF). ALVEF correlated significantly with resting RVEF (r = .45, p < .03). Although, the rate of ventricular ectopy (VPD/hr) on Holter monitoring did not correlate with resting LVEF; there was a significant linear association between ALVEF and VPD/hr (r = .47, p < .03). ALVEF did not significantly correlate with ASVR (r = .35, p < .07) but did correlate with AHR (r = .44, p < .02). In patients with IDC and stable CHF, the conclusions
are: 1) there was a wide range in ALVEF in response to IV dobutamine not related to afterload reduction, which may reflect differences in β-adrenergic receptor desensitization; 2) ALVEF was not related to established prognostic factors; and 3) ALVEF did correlate directly with RVEF and VPD/hr. Assessment of inotropic reserve by this method may prove to have a prognostic role.

Three-Dimensional Helical Computed Tomographic Angiography of the Abdominal Aorta and its Branches: An Assessment of Clinical Utility. Henry C. Hsia and Kevin W. Dickey. Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut.

Experience with three-dimensional (3-D) helical computed tomographic angiography (CTA) is presented with regard to the preoperative imaging of abdominal aortic aneurysms (AAA). Cases are presented that provide examples of obstacles to accurate and timely 3-D renderings along with strategies that were developed to circumvent them. In an attempt to examine the value of 3-D renderings in CTA interpretation, a comparison was performed between source axial images and three-dimensional renderings on the basis of their ability to provide consistent information on abdominal aortic branches. Twenty-four patients referred for elective repair of AAA were studied using CTA. Initial CTA studies were used to refine the 3-D rendering protocol. Nineteen examinations were rendered using this protocol. Source axial images and 3-D renderings were presented in separate blinded sessions to three radiologists with different levels of experience. Examinations were assessed for visualization of the celiac, superior mesenteric and renal arteries, including accessory vessels, and the presence of significant stenoses. In refining the rendering protocol, obstacles to optimal visualization encountered included unwanted contrast enhancement, eccentric stenoses, ureteral contrast, calcified stenoses, and breathing artifact. An average of 77 vessels with standard deviation (sd) of 2, including 41 renal arteries (sd = 1), were identified on axial sections versus an average of 66 vessels (sd = 4) with 40 renal arteries in 3-D renderings (sd = 1). An average of 13 arterial stenoses (sd = 8) including 11 renal lesions (sd = 6) were identified on axial sections versus an average of 8 (sd = 4) including six renal stenoses (sd = 2) in 3-D images. Interobserver agreement for axial sections was similar to that seen in 3-D imaging. Higher interobserver and intraobserver agreement was noted for identification of vessels than for identification of arterial stenoses. Despite current limitations CTA can be applied to preoperative AAA evaluation using strategies to minimize potential obstacles. Source axial and 3-D renderings should be reviewed together to maximize consistency in interpretations.

Molecular Analysis of Self Peptides that Induce Autoimmunity. Everett Hsu and Mark J. Mamula. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Maintenance of normal immunity relies on the intelligence of immune cells to distinguish self tissues and proteins from foreign pathogens. However, the presence of autoimmune diseases demonstrates a failure of the mechanisms that regulate tolerance to self tissues. The mature immune system is thus still equipped to attack self despite various developmental strategies to eliminate self-reactive T-cells. Mechanisms addressing the persistence of an autoreactive repertoire of T-cells as well as inciting agents in catalyzing the response to self are still being elucidated and are addressed in this thesis.
This study utilized murine cytochrome c (cyt c) as a model self-antigen, and particularly its carboxy terminal 81-104 used to induce a response to self. Studies were undertaken to identify the select amino acids conferring immunogenicity of this self-peptide. My preliminary studies supported the ability of the murine self-cyt c peptide 81-104 (Ms cyt c 81-104), to elicit autoimmune T-cells in normal mice. These T-cells have clearly escaped (or bypassed) the normal mechanisms that control their expression. My studies demonstrated that these autoreactive T-cells are extremely selective in their ability to bind self peptide. These studies were approached using synthetic peptide analogs that differ from the self-peptide sequence by single amino acids. Results in lymph node assays, hybrids and clones show that autoreactive T-cells specific for Ms 81-104 do not recognize peptides with the conservative 83 residue change from alanine to valine, indicating that this conformational difference is enough to render the self-peptide unrecognizable by sensitive binding of autoreactive T-cell receptors to self-murine cyt c 81-104.

In conclusion, findings of this report reveal that the difference of a single amino acid conservative residue change at 83 of Ms cyt c 81-104 can alter the specificity of binding of autoreactive T-cells. This implies a site-specific importance of certain amino acids that confer immunogenicity to self peptides. It may be postulated that conformational mimicry by exogenous proteins may initiate an autoimmune response.

Principles learned from this study have now been applied to intracellular autoantigens that are targets of lupus autoimmunity.

**Neurologic Exam and Nerve Block in Infant Rats.** Delphine Hu, Rosa Hu and Charles B. Berde (Sponsored by J. G. Collins). Department of Anesthesiology, Children’s Hospital, Boston, Massachusetts.

The purpose of the study was 1) to compare baseline peripheral nerve function in infant, adolescent, and adult rats and 2) to compare the effects of bupivacaine sciatic nerve blockade on peripheral nerve function among the various age groups.

Infant, adolescent and adult rats were evaluated for proprioceptive, thermal and mechanical nociceptive, and motor function before and after bupivacaine sciatic blockade using a modification of the neurologic exam described by Thalhammer and co-workers. Rats were injected with 0.5 percent bupivacaine at a dose of 5 ml/kg.

Mechanical and thermal nociception were present in all rats, starting from age day 1. The withdrawal reflex latency (WRL) to pinch was rapid at all ages, while the WRL to thermal stimulus increased with increasing age and lower hotplate temperatures. In contrast, the tactile placing response and hopping response were absent at birth and developed completely over the first 10 days of life. The extensor postural thrust was absent in the first two weeks of life and developed variably over the first 50 days of life. Sciatic blockade duration is shorter in infant rats than in adult rats receiving the same dose per kg. In addition, it was shown that a brief halothane general anesthetic at the time of sciatic injection in infant or adult rats does not alter the duration of blockade.

In summary, infant rats show increased sensitivity to noxious thermal stimuli and similar response to deep mechanical stimuli in comparison to adult rats. Their proprioceptive and motor responses develop over the first two weeks of life. When doses are scaled by body weight, block duration is shorter in infant than adult rats.
Evidence for Endemicity of Wild Poliovirus and Enteroviruses in a High-Risk Population in Rural Central Madagascar. Alicia A. Jacobs and Hervé Zeller (Sponsored by Jean Paul Gonzalez and Mark Cullen). Departement de Virologie, Institut Pasteur de Madagascar, Antananarivo, Madagascar.

To determine the rate of vaccination against poliovirus and the extent of circulation of enteroviruses in a country with annual reported cases of poliomyelitis, a randomized epidemiologic survey was undertaken in 354 children aged six months to five years old in a rural region of central Madagascar. A questionnaire was completed for sociodemographic assessment of participating children. Stool samples were collected for enterovirus analysis and cultured on Hep2C cell-lines, as described by WHO guidelines. There was a 45 percent rate of card-confirmed vaccination with three or more doses of oral poliovirus vaccine. Fourteen percent of all participants were positive for enterovirus. Of these, 16 percent were poliovirus strains: one wild, one Sabin and six variants. Even in this accessible region of central Madagascar, vaccination rates continue to be substandard. Severe poverty and lack of running water limited abilities to maintain good hygiene. With high rates of endemic enterovirus, including wild poliovirus, children remain at risk for contracting paralytic poliomyelitis.

Prenatal Ultrasound Biometry Combined with Umbilical and Middle Cerebral Artery Doppler Velocimetry for the Detection of Intrauterine Growth Restriction. Andrea Toulson Jeffress, Ozgur Deren, Gaurang Daftary, Theresa O'Connor and Ray Bahado-Singh. Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

We hypothesized that ultrasound estimated fetal weight (EFW) is superior to Doppler velocimetry for prediction of neonatal birth weight (BW) < 10th percentile while Doppler is more sensitive for the detection of perinatal morbidity associated with intrauterine growth restriction (IUGR).

Ultrasound biometry and Doppler velocimetry was performed on 121 consecutive patients referred for evaluation of IUGR pregnancies. The EFW was determined using standard biometric measurements and expressed as percentiles. Using color flow and pulse Doppler, resistance indices (RI) of the umbilical and middle cerebral arteries were determined. The cerebroplacental ratio (CPR), an indicator of fetal blood flow redistribution due to hypoxic stress, was calculated by dividing the middle cerebral RI by the umbilical Pd. An abnormal CPR was defined as ≤ 1. Pregnancy outcome was ascertained by reviewing maternal and neonatal charts. The Statistical Analysis System was used to analyze the data. Regression analysis was used to construct probability curves for the risk of adverse outcome based on EFW percentile and CPR.

EFW was superior to the CPR for the prediction of BW < 10th percentile, sensitivity 50.9 percent and 31.5 percent, respectively. Either an EFW below the 10th percentile or CPR ≤ 1 significantly increased the risk of BW < 10th percentile, RR (95 percent CI) 14 (4.5-44). Contrary to our hypothesis, the EFW was better than the CPR for predicting perinatal complications, sensitivity 47.7 percent and 16.3 percent, respectively. Either an EFW < 10th percentile or CPR ≤ 1 was associated with high risk of adverse outcome defined as BW < 10th percentile or perinatal morbidity, RR (95 percent CI) 29.5 (3.8-226.7) and RR (95 percent CI) 30.1 (1.8 to 513.6), respectively.

We conclude that EFW is superior to Doppler velocimetry for the prediction of BW < 10th percentile and perinatal morbidities. By combining Doppler and biometry information, we were able to more precisely estimate the risk of adverse outcome in IUGR pregnancy.
Development of an *in vitro* Technique to Study Vibration-Induced Spinal Injury. Timothy Johnson (Sponsored by Manohar M. Panjabi). Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

The etiology of most types of low back pain (LBP) remains unknown. Spinal instability (a spine is defined as "unstable" when typical patterns of motion change in response to physiologic loading) secondary to intervertebral disc injury has been hypothesized as an important cause. Many investigators have identified epidemiological evidence that associates long-term industrial vibration exposure with LBP. Long-term exposure to vibrations probably causes instability via intervertebral disc injury that manifests as LBP.

The purpose of this experiment was to develop an *in vitro* model that would allow researchers to investigate the physiologic response of human spine to vibrations. We hypothesize that by exposing cadaveric spine to certain vibrations, one may alter spinal stability via intervertebral disc injury. Theoretically, one may then quantify these alterations by detecting different intervertebral vibratory transmission patterns for the pre- and post-injury states.

Several mechanical apparatus capable of transmitting vibrations to human cadavetic lumbar spine were developed and constructed. Each apparatus was rigidly attached to an epoxy-mounted, 3 vertebra lumbar-spine segment. All soft tissue was dissected from each specimen leaving only the ligamentous spine with vertebrae, discs and attached ligaments. Specimens were vibrated at predetermined amplitudes, frequencies and time intervals to assess the adequacy of each model. Electronic markers secured to the vertebral bodies were used to track the three-dimensional motion of each vertebral segment with the aid of the Optotrak Motion Measuring System, and a microcomputer-assisted digital data acquisition unit. Software was written to perform data analysis.

Several inadequate models were developed before obtaining one that was capable of providing uniform vibrations over the desired range of frequencies and amplitudes. This model consisted of a long steel crankshaft adjoining an electric-motor-powered wheel on one end and the test specimen on the other. This apparatus was capable of providing consistent cyclical motion at adjustable amplitudes (3 to 12 mm at 1 mm intervals) and frequencies (0 to 32 Hz at 2 to 3 Hz intervals) of vibration. Once the model was obtained, a multi-staged protocol was developed to systematically injure and evaluate the stability of each specimen before and after injury. One specimen has been tested with this model.

Further specimen testing using the aforementioned study protocol and vibration producing apparatus need to be conducted before conclusions may be drawn regarding the vibration-induced instability hypothesis.

Lack of Polymorphisms at the 5′ end of the c–MYC Oncogene in Nasopharyngeal Carcinoma. Raymond W. Kam (Sponsored by James F. Jekel and William C. Summers) Departments of Epidemiology and Public Health, and Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut.

Nasopharyngeal carcinoma (NPC) tumor biopsies from southern China were analyzed for the presence of c–MYC oncogene polymorphisms in the 5′-region of the c–MYC gene which is known to be hypermutable in endemic Burkitt lymphoma (eBL). This region was examined because both NPC and eBL are strongly associated with elevated Epstein-Barr virus titres. DNA from tumor biopsies and blood samples were isolated from newly diagnosed cases, and the 5′-region of the c–MYC gene including the 5′-flanking region, first exon, and a portion of the first intron, were amplified using the polymerase
chain reaction. Amplified DNA was purified by gel electrophoresis followed by an isolation process using silica beads in the presence of a chaotropic salt. The samples were then eluted into a standard buffer and digested with the following restriction enzymes: EcoRI, HindIII, PstI, PvuII, SacI, and XbaI, all of which are known to reveal polymorphisms in eBL cell lines and biopsies. No polymorphisms were detected in the four NPC samples examined, which suggests that the somatic mutations present in eBL are not present in NPC with the same frequency.

**Targeting of Calcitonin Gene Related Peptide Expression to the Beta Cells of Non-obese Diabetic Mice Prevents Insulin-Dependent Diabetes Mellitus.** Armen Khachatryan, François Palluault, Sylvie Guerder, Gilbert Cote, Isabelle Millet, Karine Valentijn, Richard A Flavell, Agnès Vignery. Departments of Cell Biology, Orthopaedics and Rehabilitation, and Immunobiology, Yale University School of Medicine, New Haven, Connecticut.

To investigate whether the immunosuppressive neuropeptide calcitonin gene related peptide (CGRP) was a potential candidate for tissue-specific gene therapy, we engineered Non-Obese Diabetic (NOD) mice to produce CGRP in beta cells by placing the modified calcitonin gene under the control of the rat insulin promoter. CGRP inhibits the production of cytokines by Th1 cells, which have been implicated in the pathogenesis of type I diabetes. Three transgene-positive mouse lines were obtained, two of which express immunoreactive CGRP in beta cells (NOD-CGRP mice). Isolated islets from one of these two transgene-positive founders produced active CGRP while islets from transgene-negative mice did not. The production of CGRP by beta cells prevents IDDM in male, and reduces its incidence by 63 percent in female NOD mice. This immunosuppressive effect of CGRP is due to a local effect and not a systemic effect of CGRP on peripheral lymphoid organs as no difference was detected between NOD-CGRP and NOD mice lymph node, spleen and thymus cells by either fluorescence-activated cell sorter analysis or proliferative response to stimulation by antigen, alloantigen or anti-CD3. CGRP partially prevented peri-insulitis in NOD-CGRP mice whose lymphoid cell composition was similar to that of NOD mice, further suggesting the local immunoregulatory effect of CGRP. These data suggest that CGRP is a potential therapeutic molecule to prevent or treat diabetes and possibly other diseases and conditions in which immune cells are involved. These data also suggest that endogenous CGRP concentrated in sensory nerve endings may regulate locally in tissues the immune response, further strengthening the importance of the functional neuro-immune link.

**Quantification of Estimation Error in Oscillometric Blood Pressure Monitoring Systems and Proposed Modification of the Current Algorithm to Enhance Estimation Accuracy in Humans.** Anwar M. Khan (Sponsored by Conrade C. Jaffe). Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut.

Oscillometry and auscultation constitute two of the most widely used non-invasive methods of measuring the blood pressure indirectly in the medical practice. Of these two techniques, auscultation is particularly more susceptible to measurement error in situations that involve noisy background, very low blood pressures or measurements in children and infants; such situations warrant the use of oscillometry as the preferred non-invasive technique. In the oscillometric technique, as the cuff pressure is decreased...
systematically, measurable oscillations are generated in the cuff pressure that begin as small excursions, reach a maximum and then decrease again. The oscillometric technique utilizes the information of this crescendo-decrescendo behavior to estimate the systolic and diastolic pressures in conjunction with factory-preset parameters referred to as systolic ratio (SR) and diastolic ratio (DR). However, the values currently used for these ratios are derived from animal models and have not been analyzed adequately in humans. The objective of this study was to quantify the oscillometric estimation error in humans, to investigate any determinable relationship between the error and the algorithmic parameters, and to propose modifications to the current algorithm to optimize estimation accuracy. Using a multi-channel custom-designed data acquisition system, we recorded simultaneous direct and oscillometric blood pressure data waveforms from 25 hospitalized patients aged 24 to 82 years (mean 52 years). These data were processed and analyzed using several custom-written and commercially available software tools. The use of currently accepted parameters consisting of SR = 0.5 and DR = 0.8 underestimated the systolic pressure by −5.1 mm Hg (range −31.7 to 11.2) and overestimated the diastolic pressure by +7.3 mm Hg (range −5.5 to 18.5), respectively. Our analysis suggests that mathematical relationships between the mean systolic estimation error vs SR and mean diastolic estimation errors vs DR do exist and can be expressed as Systolic Error = 37.41 − 85.01 (SR), and Diastolic Error = −19.25 + 33.18 (DR). These equations imply that setting the SR = 0.44 and DR = 0.56 would theoretically reduce the systolic and diastolic estimation errors to zero.

The Yale Experience with Early Stage Bilateral Breast Carcinoma: Treatment Comparisons and Prognosis. David H. Kim and Bruce G. Haffty. Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this investigation is 1) to determine the prognostic effect of the development of a second breast cancer on the survival of a woman with early stage breast cancer and 2) to assess the use of bilateral lumpectomy and radiation therapy as an alternative to bilateral mastectomy in women with early stage bilateral primary breast carcinoma. The charts of all patients with the diagnosis of breast cancer treated with conservative therapy at Yale-New Haven Hospital prior to 1993 were retrospectively reviewed to identify patients with bilateral disease. A total of 50 patients identified as having bilateral breast cancer conservatively treated served as the index population (BCT). A group of 984 patients with unilateral breast cancer treated with conservative therapy during the same time period served as the first control group (UCT). A second control group was comprised of 42 patients with early stage bilateral breast cancer presenting during the same time interval treated with bilateral mastectomy (BMAST). Patients with locally advanced disease in either breast or those patients treated exclusively for lobular carcinoma in situ in either breast were excluded from analysis. No statistically significant differences were noted between the three groups with respect to age, stage, nodal status, adjuvant systemic therapy use, or ER/PR status. Survival comparisons were made between the three groups with respect to overall, locoregional relapse-free, distant relapse-free, and no evidence of disease (NED) survival. As noted below, there were no statistically significant survival differences between the study groups.
Survival at five years.

| Overall Survival | Disease-Free Survival | Relapse-Free Survival | NED Survival |
|------------------|-----------------------|-----------------------|-------------|
| BCT 91 percent   | 91 percent            | 93 percent            | 80 percent  |
| UCT 88 percent   | 89 percent            | 94 percent            | 84 percent  |
| BMAST 81 percent | 74 percent            | 93 percent            | 71 percent  |

We conclude that 1) women with early stage bilateral breast cancer carry a prognosis equivalent to patients with early stage unilateral disease and 2) bilateral breast conserving therapy is an acceptable alternative to bilateral mastectomy in stage I and II bilateral breast cancer.

Quantitative Three-Dimensional Anatomy Of the Upper Cervical Spine. Timothy J. Kinkead (Sponsored by Manohar Panjabi). Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

In this study, the three-dimensional quantitative anatomy of the occiput (C0), atlas (C1) and axis (C2) was determined. The three-dimensional coordinates of various marked points on the surface of each osseous structure were measured using an Optotrac morphometer. From these coordinates linear dimensions and surface areas of clinically important vertebral components were calculated. The mean and standard deviations are presented here and compared with the results of previously published data.

Acute Exacerbations of Asthma in Children: Relapse after Treatment in the Emergency Department. Barbara L. Klock (Sponsored by Jean E. Klig). Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

This study retrospectively compared children with asthma who relapsed to the Emergency Department (ED) after treatment with those who did not relapse to identify possible differences in the acuity of asthma symptoms and treatment provided. A scoring system to identify patients at risk for relapse was evaluated. A total of 254 children were enrolled; 127 patients who relapsed within fourteen days of initial treatment (R) were matched with 127 patients who did not relapse (NR). R children had more prior hospitalizations for asthma (p < .01) and more courses of corticosteroids in the past (p < .01) than NR children. Onset of respiratory symptoms and medications taken prior to the ED were similar for both groups. Heart rate, respiratory rate and oxygen saturation did not differ between the R and NR groups on admission to the ED and at discharge. Nebulized medications received in the ED were similar for the two groups. Corticosteroids were administered with equal frequency to both groups (p = NS). Total scores stratified into three groups corresponding to mild, moderate and severe asthma exacerbations were highly associated with these outcomes (p < .0004). It was concluded that children who relapse following treatment for an acute exacerbation of asthma may appear clinically similar to children who do not relapse and corticosteroid treatment in the ED may not prevent early
relapse for a group of these patients. Most children who relapse complain of continued wheezing (93 percent) and return within seventy-two hours of discharge (72 percent). A scoring system comprised of features of the past asthma history and present exacerbation of asthma may identify children at greatest risk for relapse.

Factors Influencing the Specialty Choice of Physicians Trained at Yale-New Haven Hospital: 1928-1994. David Matthew Krol (Sponsored by Edwin Cadman). Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study was to examine the factors that influenced the specialty choices of physicians who completed or are completing their training in a residency affiliated with the Yale-New Haven Hospital. An examination and comparison of those physicians graduating from medical school during the decades between 1920 and 1990 was made. A questionnaire was mailed to all physicians noted to have participated in a residency at Yale-New Haven Hospital from 1928 to 1994 in the American Medical Association Physician Master File. The questionnaire included a group of 36 factors posited to be influential in specialty choice. Each factor was rated by the physicians on a 7-point Likert scale from a very negative to very positive influence. Examination of the results included an examination of specialty groups as well. The results of this study show that the group of positive influences on specialty choice was similar in each of the decades from the 1920s to the 1990s, between sexes and when school status was examined. These influences included "altruistic" factors such as interest in helping people and opportunity to make differences in people's lives and "specialty characteristics" factors such as intellectual content of the specialty, challenging diagnostic problems and diversity in diagnosis and treatment. There has been much literature on medical student specialty choice and how to predict and influence their choices. From this study, it would seem that the best way to influence specialty choice is to change the inherent characteristics of the specialties, students and patients. This, in the author's opinion, is a very difficult thing to do and unless these influential factors are kept in mind when making workforce policy there will be many physicians practicing specialties that do not fit them.

Effect of Manipulating Intra-amygdala Levels of cGMP on Fear Conditioning. J.W. Landsberg. Departments of Psychology and Psychiatry, Yale University, New Haven, Connecticut.

Long-term potentiation (LTP) describes the persistent, activity-dependent increase in synaptic efficacy which has been seen in many vertebrate brain areas both in vitro and in vivo and is considered by many to be a promising model of learning and memory. Recent in vitro studies of the CA1 region of the rat hippocampus have implicated cyclic guanosine 3', 5'-monophosphate (cGMP) as an important second messenger in the formation of LTP. LY-83583, an inhibitor of cGMP production, was shown to block LTP, while addition of 8-Br-cGMP, the membrane permeable cGMP analogue, produced LTP. We conducted the following experiments to investigate the effect of intra-amygdala infusion of LY-83583 and 8-Br-cGMP on fear conditioning, an example of in vivo learning, as measured behaviorally by fear potentiated startle in male Sprague-Dawley rats. Bilateral intra-amygdala infusion of 2.5 nmol LY-83583, 6 min prior to 30 light-shock (0.6 mA) pairings, failed to block the acquisition of conditioned fear. Bilateral intra-amygdala infusion of 50 nM 8-Br-cGMP, 6 min prior to 30 light-shock pairings, failed to enhance the acquisition of either weak fear conditioning or strong fear conditioning. Overall, we were not able to
manipulate the acquisition of fear potentiated startle by intra-amygdala infusion of these two agents recently shown to impair and enhance LTP in vitro.

Issues of Fairness in Physician Workforce Reform. Clara N. Lee (Sponsored by Sherwin B. Nuland and Theodore Marmor). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

This study sought to encourage attention to issues of fairness in physician workforce reform and to evaluate the current workforce debate for its treatment of fairness issues. It was hypothesized that the literature on workforce reform contains only implicit moral justifications for intervention into physicians' lives and that the validity of many of those justifications has not been examined. A theoretical framework was devised to explicate fairness issues raised by workforce policies. The medical and policy research literature, proposals by private and public research organizations and communications from medical professional societies were subjected to content analysis to identify implicit moral justifications for reform. Four implicit justifications for reforms were found in the literature, including, the public subsidy of physicians' training, income and knowledge, the magnitude of health care costs, resentment of physician incomes and erosion of physician respect and society's responsibility to guarantee health care. The literature did not question the validity of these justifications. Although none of the accounts fully addressed all theoretical concerns about fairness, society's responsibility to guarantee health care provided the most complete justification of reforms. Remaining issues of fairness and directions for future research were established.

Photochemotherapy of Vascular Cells with 8-Methoxypsoralen and Visible Light: A Novel Approach to the Prevention of Post-Angioplasty Restenosis. David M. Lee, Francis P. Gasparro, and Bauer E. Sumpio. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The purpose of the present study was to evaluate the effects of photochemotherapy with 8-methoxypsoralen (8-MOP) and visible light (447 nm) on vascular cells in vitro. It was hypothesized that this treatment regimen could selectively inhibit smooth muscle cell proliferation and might thus represent a novel approach to the prevention of post-angioplasty restenosis.

Bovine aortic smooth muscle cells (SMC) and endothelial cells (EC) were cultured in cell wells, incubated with 1, 20 or 50 µg/ml 8-MOP, and then exposed to 12.0 J/cm² 447 nm visible light. Control cells were wrapped with aluminum foil so as to prevent 8-MOP photoactivation. Studies were then undertaken to ascertain the effects of these treatment regimens on SMC and EC proliferation, migration, morphology, size and photoduct formation.

Experiments on SMC proliferation revealed no significant effect at 1 µg/ml 8-MOP, while 20 µg/ml produced a transient, reversible inhibition, and 50 µg/ml resulted in cell death. Experiments on EC proliferation also revealed no significant effect at 1 µg/ml 8-MOP; however, a transient, reversible inhibition was seen at both 20 and 50 µg/ml 8-MOP. Further experimentation revealed that photoactivated 8-MOP does not inhibit cell migration. Morphology studies demonstrated both size and phenotype changes induced by higher dosages (20 and 50 µg/ml) of 8-MOP. Cell size measurements corroborated the cell enlargement observed in the morphology studies. Followed over time, EC revealed a return to baseline sizes while SMC remained enlarged. Finally, studies on photoduct
formation revealed the formation of comparable numbers and types of photoadducts between SMC and EC.

These experiments demonstrate that while 8-MOP photoactivated with 447 nm visible light can reversibly inhibit the proliferation of both SMC and EC in a dose-dependent fashion, SMC are more sensitive to the treatments than EC. The rapidly reversible inhibition observed in EC proliferation bears clinical significance, as previous studies have demonstrated that an intact endothelium contributes to the inhibition of SMC proliferation. As SMC proliferation constitutes a major mechanism by which restenosis after angioplasty occurs, the results of this study suggest that photochemotherapy with 8-MOP and visible light may represent a novel approach to the prevention of post-angioplasty restenosis.

The Expression of Angiogenic Growth Factors and Extracellular Matrix Proteins in Central Nervous System Vascular Malformations. Jiyon Lee, Issam Awad and David Rothbart. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Arteriovenous malformations (AVM) and cavernous malformations (CM) are two clinically significant types of vascular malformations of the central nervous system. Though both have been extensively described in the neurosurgical literature with regard to their clinical and biological behavior, diagnosing imaging characteristics and surgical considerations, very little investigative effort has been directed at exploring the molecular pathogenesis and evolution of these lesions. The possible role of angiogenic growth factors in conjunction with extracellular matrix proteins in their genesis has not previously been considered. In this study, we subject twelve neurosurgical specimens (7 AVM, 5 CM) to a battery of immunostaining for two known angiogenic growth factors: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF); and selected matrix proteins: lamin (LN), fibronectin (FN), collagen type IV (CoIV) and alpha smooth muscle actin (ASMA). VEGF was expressed by all twelve lesions, localized primarily in the subendothelium and perivascular tissue. Basic FGF was faintly expressed in four of seven AVMs and four of five CMs in the media of AVM vessels, and the subendothelial layer and intercavernous matrix of CMs. The pattern of angiogenic growth factor expression was correlated with the expression of matrix proteins. LN was not expressed in any of the CMs, confirming previous reports from our laboratory. However, FN was more prominent in CMs. CoIV and ASMA expression occurred in every lesion. We conclude that angiogenic growth factors are expressed in both AVMs and CMs on the CNS. The pattern of immunostaining suggests diffuse activation of angiogenesis without specific relation to individual vessel types or recent clinical hemorrhage while the pattern of matrix proteins expression suggests that CMs lack mature vessel wall elements and have an “angioproliferative” matrix milieu as compared to AVMs. We hypothesize that AVMs and CMs may represent “dysvascuologenesis” and “dysangiogenesis” respectively, both concepts discussed in depth in this thesis. Clarifying the role of angiogenesis in vascular malformations may provide insight into their pathogenesis and suggest novel strategies for modification of their behavior.
Intracellular and Extracellular pH Modulates Epileptiform Activity in the Rat Hippocampus. Junhee Lee and Bruce R. Ransom. Department of Neurology, Yale University School of Medicine, New Haven, Connecticut.

To explore the role of pH in modulating epileptic activity, experiments were conducted using hippocampal slices treated with the GABA$_A$-blocker bicuculline: such slices produce periodic, brief, epileptiform discharges very similar to the interictal discharges detected in epileptic brain in vivo. Double-barreled, ion-sensitive microelectrodes (4 to 6 µm tip diameter) were used to simultaneously record field potentials and either extracellular pH (pH$_e$) or [K$^+$] in the slices.

The incidence frequency of spontaneous epileptiform discharges proved highly sensitive to changes in pH effected via changes in pCO$_2$: pH$_e$ acid shifts as small as 0.05 pH units were associated with an average frequency decrease of 26 ± 3 percent (SE; N = 4). Epileptiform discharges were reversibly abolished by a mean pH$_e$ acid shift of 0.20 ± 0.03 pH units (SE; N = 4). These effects showed no consistent correction trends, even with pH$_e$ shifts less than 0.1 units maintained for greater than 45 min. Altering pH$_e$ through changes in bath [HCO$_3^-$] had steady-state effects indistinguishable from those of pCO$_2$-induced pH changes. Experiments using K$^+$-sensitive microelectrodes indicated that these pH effects were not mediated by changes in extracellular [K$^+$]. The pH effects appeared to be mediated, at least in part, intracellularly: addition of weak acids and bases known to cause intracellular acidification and alkalinization were found to suppress and augment (respectively) spontaneous activity.

The data presented here not only have therapeutic implications, but also provide insight into the self-terminating mechanisms of epileptic seizures: given that neural activity-induced extracellular acid shifts of > 0.1 pH units have been observed in the mammalian CNS, seizure-induced pH shifts may very well act as a negative feedback inhibitor (Ransom, 1992).

Astrocyte Gap Junctions and their Contribution to Non-synaptic Epileptogenesis. Sunghoon Lee (Sponsored by Anne Williamson). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

This study investigates the possible involvement of glial cells in epilepsy. New and exciting evidence for inter-glial signaling by intercellular calcium waves led us to investigate the role of gap junction coupling between astrocytes. Our hypothesis was that inter-astrocytic calcium waves propagating through gap junctions connecting neighboring astrocytes could influence the excitability of regions distant from the original sites of excitation.

We used a novel technique called Fluorescence Recovery after Photobleach (FRAP) to examine functional dye coupling in cultured astrocytes and determined that rat astrocyte coupling is region-specific. We then hypothesized that comparable region-specific astrocyte coupling may be related to the regional specificity of epileptogenic thresholds observed clinically. We tested this hypothesis using human astrocytes cultured from five different substrates associated with intractable epilepsy. We found a substrate-specific correlation in the frequency of intercellular calcium waves and the extent of dye coupling. Especially notable finding was found in peri-tumoral cortex which showed “hyperexcitability” in intraoperative ECoG (in vivo), a high frequency of intercellular calcium waves by calcium imaging, and a high degree of dye coupling by FRAP.

These findings in human astrocytes led us further to hypothesize a role for glial coupling as a possible non-synaptic mechanism of epileptogenesis and seizure propagation: a
pathological enhancement of gap junction coupling may allow for an enhancement of the frequency inter-astrocytic signaling. Therefore, we studied the low Ca\(^{2+}\)/high K\(^+\) model of non-synaptic seizures using hippocampal slices from rats. In this model, prolonged seizure-like events occur spontaneously and propagate through the tissue. We found that the phenomenological characteristics and pharmacological parameters of these events support the hypothesis that glial gap junction coupling may be important in allowing the propagation of this type of epileptic activity in cortical structures.

**Stimulation of Glucose-Dependent Insulinotropic Polypeptide, Not Glucagon-Like Peptide-1, after a Modest Oral Glucose Load in Young Subjects.** Charles J. Limb, William V. Tamborlane, Robert S. Sherwin, Fran Rife, Raymond Pedersen, Thomas O'Dorisio and Sonia Caprio. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Oral glucose induces a greater insulin response than intravenous glucose, a difference apparently due to the secretion of gut factors ("incretins"). Interpretation of such studies in human subjects is limited, however, because of differences in glucose profiles. To overcome this obstacle, we studied 10 young subjects (ages 15 to 28) using the hyperglycemic clamp with and without late ingestion of oral glucose. In both studies, glucose was acutely raised by 125 mg/dl above fasting values by the infusion of intravenous glucose and maintained at this level for 180 minutes. During the experimental study, but not the control, each subject ingested oral glucose (30 g) at 120 min. The glucose infusion was adjusted to maintain the plasma glucose plateau. Plasma insulin responses were nearly identical during both studies until oral glucose was added. After oral glucose both plasma insulin (p < .01) and C-peptide (p < .001) levels sharply increased by 45 to 55 percent above control values, indicating a potentiation of insulin secretion rather than decreased hepatic extraction of insulin. Plasma gastric inhibitory polypeptide (GIP) levels increased significantly in response to oral glucose whereas plasma levels of glucagon-like peptide-1 were surprisingly not affected. The time courses of the rise in plasma GIP and insulin were nearly identical. Conclusion: The GIP response to a modest oral glucose load may play an important physiologic role in glucose-stimulated insulin secretion in healthy young subjects.

**Adjuvant Analgesics in Cancer Pain and the Role of Metoclopramide.** Rebecca Woolsey MacLean (Sponsored by Ann Berger). Departments of Anesthesiology and Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

This research project was conducted to evaluate the safety and efficacy of metoclopramide for the relief of visceral cancer pain. Metoclopramide is used worldwide for gastrointestinal motility disorders and nausea. It has recently been shown to relieve pain, but has not been studied as an adjuvant analgesic in cancer. A randomized, double-blind, crossover trial comparing metoclopramide, 40 mg daily for one week, to placebo was conducted from July to December, 1995. Patients were accrued through the Yale-New Haven Hospital outpatient oncology clinics. Primary outcome measures were visual analogue pain scores and daily analgesic requirements as recorded by patients in "pain diaries." Twenty-one patients with visceral pain and cancer were evaluated for participation. Sixteen of 21 failed to meet inclusion criteria. The most common reasons for exclusion were non-consent and concurrent treatment with palliative radiotherapy or celiac plexus blockade for pain. Five patients were enrolled, and one completed the protocol in its
entirety. Reasons for discontinuation included two cases of mechanical small bowel obstruction. Quantitative analysis of the results of this project were seriously compromised by the high attrition rate. Two cases of sedation and one of restlessness were attributable to metoclopramide. Recommendations for future research include evaluating a larger patient population and systematically assessing quality-of-life measures to better determine the clinical relevance of metoclopramide in the care of people with pain and advanced cancer.

Perceptions of Vulnerability and Fears of AIDS: A Survey of Elementary School Children. John S. Maypole, Linda O’Hare, Donald R. Showalter, Domenic V. Cicchetti, and David J. Schonfeld. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Few investigations have addressed the fears and perceptions of vulnerability to HIV infection among elementary school children. Understanding and characterizing these fears and perceptions may inform efforts to eliminate unnecessary fears arising from misconceptions and to foster appropriate perceptions of risk in this population. Children (N = 361; grades K to 6; 57 percent black, 24 percent Hispanic, 19 percent white; 52 percent female) attending four public schools in New Haven, Connecticut were interviewed using a standardized, semi-structured interview (ASK, AIDS Survey for Kids) that included questions about children’s fears and perceptions of vulnerability to AIDS. Responses to each question about fear or vulnerability were scored according to their content using criteria developed by the principal investigator. Analyses examined the nature of these responses as well as the pattern of responses of younger (grades K to 3) versus older (grades 4 to 6) children. In response to the questions about perceptions of vulnerability, a large majority of children (86.4 percent) believed that anyone could get AIDS, and 79.8 percent of all children responded they thought themselves at risk of getting the disease. Significantly fewer younger children (74.2 percent) thought they themselves could get AIDS when compared to older children (86.5 percent). Fear of having AIDS was more prevalent in younger children (61.1 percent) than in older children (46.0 percent), yet few children (8.1 percent of the younger group and no children in the older group) actually thought they had AIDS on follow-up questioning. The majority of those who worried about getting AIDS mentioned the lethality of the disease. Though older children more often to acknowledged that they could get AIDS, 20 percent of younger children but only 2.5 percent of older children thought they will get AIDS in the future. Older children may be more apt to exercise denial in their perceptions of personal risk of getting AIDS. The implications of these findings for educational interventions highlight the need for developmentally-appropriate curricula. Younger children may need education to address excessive fears of getting AIDS, while older school-age children may benefit from instruction to realign their knowledge of vulnerability and perception of personal risk for contracting the disease.

Effects of Background Clutter on Scatterer Number Density Estimation. Martin L. Mayse and John Gore. Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut.

This paper proposes an ultrasonic model of human tissue in which a set of relatively strong, sparsely spaced acoustical scatterers is superimposed on a background clutter of relatively weak, densely packed scatterers. The effect of background clutter on the estimation
of the number of sparse scatterers per unit volume (the scatterer number density) is evaluated. When background clutter is ignored, it is shown the estimator \( F_m F_r / (K-3) \) where \( F_m \) and \( F_r \) are known constants and \( K \) is the kurtosis of the backscattered signal, is biased by a multiplicative factor of \((1 + CSR^2)\). CSR\(^2\) (read Clutter-to-Signal Ratio squared) is the ratio of scattering intensity of the background clutter to the scattering intensity of the sparse scatterers. However, it is also shown that when the value of CSR\(^2\) is known, the effect of background clutter can be removed, yielding an unbiased estimator of the number density of the sparse reflectors.

Five tissue-mimicking phantoms were constructed, each with a sparse scatterer number density of 70/cm\(^3\), but CSR\(^2\) values of 0, 0.35, 0.75, 1.1, 1.7. Without correction for the effects of background clutter, estimates of the number density of the sparse scatters were 64, 117, 179, 259 and 418/cm\(^3\) respectively. In the extreme (CSR\(^2\) = 1.7) this estimate varies nearly 50% percent from the true value. On the other hand, with \textit{a priori} knowledge of CSR\(^2\), the sparse scatterer number density estimates were independent of the value of CSR\(^2\) and averaged 62.1/cm\(^3\), a variation of only 11% percent from the true value.

**The Value of Lateral Chest Radiographs in the Assessment of Bone Density and the Detection of Osteopenia.** Monica A. Medynski. Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut.

The reading of "osteopenia" on a lateral chest radiograph, using a high K\textsubscript{p} technique, does not correlate with the presence of osteoporosis as demonstrated on bone biopsy. Thirty-five lateral chest films of patients with identified osteoporosis through a bone biopsy and 26 lateral chest films of patients with no evidence of osteoporosis on bone biopsy were coded. All the radiographs were reviewed by three radiologists, two chest and one bone specialist, who were asked to use specified criteria for the detection of osteopenia. The data were analyzed for interobserver and intraobserver variability using weighted kappa. Odds ratios were calculated to see if any of the criteria we used in evaluating lateral films could correctly predict the presence of osteoporosis. The radiologists seemed relatively consistent in their evaluation of osteopenia. Weighted kappa comparing viewing one and viewing two were equal to 0.60, 0.58 and 0.60 for the three readers, representing "moderate/acceptable" agreement. As is usual, there was less agreement between the readers: the interobserver variability fell into the range of "fair/moderate" with weighted kappa for general assessment of osteopenia equal to 0.40, 0.45 and 0.53. None of the criteria seemed to reliably predict the presence of osteopenia based on the calculation of odds ratios with a 95% percent confidence interval. In conclusion, our results prove osteopenia in the thoracic spine may not be consistently detected on lateral chest radiographs, at least with this physician sample. The fact that one radiologist was capable of detecting osteopenia suggests that there may be as yet an inarticulated template that corresponds to osteopenia of the spine. This has major implications for radiology education. Further efforts to either articulate the template or provide multiple shared experiences of film interpretation to transmit the template are warranted.
Congestive Heart Failure in an Elderly Population: Predictors of Hospitalization and Mortality. Jeremy C. Miller, Christianna Williams, and Harlan M. Krumholz. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Congestive heart failure (CHF) is one of the most common sources of morbidity and mortality among individuals aged 65 years and older. The purpose of this study was to identify independent predictors of hospitalization and mortality secondary to CHF in the elderly.

The New Haven cohort of the Established Populations for the Epidemiologic Study of the Elderly (EPESE) program was screened for incident cases of decompensated CHF. Detailed clinical information was collected retrospectively and was merged with prospective demographic, psychosocial and functional data from the EPESE cohort to create a comprehensive database. Multiple logistic regression was used to calculate odds ratio (OR) and 95 percent confidence intervals (95 percent CI).

There were 295 confirmed cases of CHF among the 2,812 cohort members. Annual incidence was 10.7/1000 for men, and 10.3/1000 for women. The most powerful significant independent risk factors for hospitalization for CHF were diabetes (OR 1.79) and history of myocardial infarction (OR 1.78), although age (OR 1.03 per year), severe exertional dyspnea (OR 1.48), hypertension (OR 1.33) and lack of emotional support (OR 1.40) were also predictive.

The strongest significant independent predictors of mortality at one year among cases of CHF included unknown left ventricular ejection fraction (LVEF) (OR 4.64), LVEF < 55 percent (OR 3.02), and myocardial infarction as precipitant of heart failure (OR 2.25). Other significant predictors of mortality included older age (OR 1.05 per year), male sex (OR 2.22) and social connections ≥ 1 (OR 0.43).

In conclusion, demographic and psychosocial characteristics, in addition to cardiovascular factors, are substantially important predictors of the risk for hospitalization and mortality secondary to CHF in the elderly. Better understanding of elderly patients at risk for CHF and subsequent mortality is an essential step in designing appropriate medical and social interventions for this increasingly important public health problem.

Regulation of CD44 Expression in the Human Myelomonocytic Cell Line (THP-1) and the Pattern of CD44 Expression in Granulomatous Diseases. Leigh H. Miyamoto, Robert Homer and Carolyn L. Rochester, Departments of Internal Medicine and Pathology, Yale University School of Medicine, New Haven, Connecticut.

CD44, a transmembrane glycoprotein expressed on most cells of hematopoietic origin, is the principal cell surface receptor for hyaluronic acid (HA) and has also been implicated in lymphocyte homing, T-cell activation, and tumor metastasis. The purposes of this study were to 1) examine the pattern of CD44 expression within tissue specimens from patients with various granulomatous diseases including sarcoidosis, tuberculosis and Crohn's colitis and 2) characterize the constitutive expression of CD44, as well as induced CD44 expression elicited by lipopolysaccharide (LPS, a polyclonal activator of monocytes), and the cytokines interferon-gamma (IFN-γ) and interleukin-4 (IL-4) in the human THP-1 myelomonocytic cell line. The techniques of cell culture, RNA isolation, northern analysis, flow cytometry and immunohistochemistry were utilized. We demonstrate that CD44 protein expression is prominent in a membranous pattern on macrophages/histioocytes and giant cells within granulomas from a variety of tissues (skin, lung, lymph node, intestine) derived from patients with granulomatous diseases. We also show that THP-1 cells constitutively express small amounts of CD44 protein and that LPS upregulates TBP-
1 cell expression of CD44 mRNA and protein in a dose- and time- dependent manner. In contrast, stimulation of cells with IFN-γ causes only weak augmentation of CD44 expression in these cells. IL-4, a cytokine known to downregulate a variety of monocyte functions and to modulate expression of cell surface adhesion molecules, downregulates the LPS-induced increase in cellular expression of CD44 mRNA, but does not alter constitutive expression of CD44 mRNA. These results demonstrate that soluble inflammatory mediators can modulate CD44 expression in the TBP-1 myelomonocytic cell line and support the hypothesis that CD44 may play a role in granuloma formation.

**Human Immunodeficiency Virus and Sentenced Men in Connecticut: An Anonymous Study of Seroprevalence, Risk Factors and Detection.** Farzad Mostashari and Frederick Altice. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

We intended to determine the seroprevalence and risk factors of HIV infection, and characterize the efficacy of voluntary counseling and testing (VC/T) in detecting HIV infection among a cohort of sentenced men. Medical intake forms, supplemental risk behavior assessments and laboratory information was anonymously linked to discarded sera for 975/999 (98 percent) consecutive sentenced men in Connecticut.

1. HIV seroprevalence was 6.1 percent.
2. Independent risk factors for HIV infection were injection drug use (IDU) (OR = 8.9, p < .001), Black race (OR = 5.5, p < .001), Hispanic ethnicity (OR = 3.4, p < .01), a history of psychiatric problems (OR = 3.1, p < .01) and a history of STDs (OR = 2.2, p = .02).

3. A minority of HIV-positive individuals (10 percent) reported no risk factors for HIV infection other than use of crack cocaine. There was a high level of laboratory abnormalities among symptomatic and asymptomatic HIV-positive men, in particular low white cell counts and low albumin levels.

4. Most HIV-positive inmates (72 percent) knew of and reported their HIV status.
5. Diagnosed HIV-positive men had higher rates of condom use with their primary partners compared to undiagnosed HIV-positive men or HIV-negative men.

6. The prison-based VC/T program's efficacy was limited by non-adherence to referral criteria, and lack of counseling resources, however, nearly all (90 percent) HIV-negative and HIV-positive inmates accepted HIV testing.

In conclusion, there are high rates of HIV infection in Connecticut prisons, mostly related to injection drug use and minority status. Voluntary counseling and testing can be a less coercive, and effective, alternative to mandatory testing and can be improved through increased adherence to referral criteria, improvement of referral criteria (e.g., through addition of laboratory "flags" or crack use) and increased counseling resources.

**Successive Desensitization: A Method for Low-Pain Delivery of High Doses of Capsaicin to the Human Tongue.** Wolffé Nadoolman (Sponsored by Linda M. Bartoshuk). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study was to test the hypothesis that the human tongue could be made insensitive to the pain of capsaicin by a series of increasing topical doses. Capsaicin is the chemical principally responsible for the burning sensation associated with eating chili peppers.
The method was to compare burn ratings of 10 healthy volunteer adult human subjects in the undesensitized state to burn ratings from those same subjects after the successive desensitization procedure. In the first stage, subjects rated the pain of solutions applied to the tongue with concentrations of 1, 10 and 100 parts per million (ppm). The following day, the successive desensitization procedure was carried out by applying capsaicin concentrations graduated in 1/4 log increments (1, 1.8, 3.2, 5.6, 10, 18, 32, 56, 100 ppm). Each subject rated the burn associated with a brief probe of the benchmark concentrations of 1, 10 and 100 ppm immediately after they reported the fading of the burning sensation from the application of those concentrations. The ratings of each subject after the experimental procedure were then compared with that same subject’s ratings before the procedure. In this way, each subject was his or her own control. Burn intensity ratings were normalized to allow comparison between subjects.

Two-way analysis of variance for the paired before-and-after ratings of 1, 10 and 100 ppm showed a statistically-significant (p < .001) reduction in perceived pain after each subject underwent the successive desensitization procedure. Planned comparisons for the student’s t-test were also significant (for 1 ppm, p < .02; 10 ppm, p < .002; 100 ppm, p < .001). The demonstrated effectiveness of this procedure could provide the basis for certain clinical applications in which the burning sensation associated with capsaicin prevents its therapeutic use.

**Analysis of in vivo Tissue Expander Fluid Composition.** Richard A. Nahouraii (Sponsored by Richard J. Restifo). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The ability of the electrolytes of normal saline to diffuse through tissue expanders implanted in rats was examined. Eleven Sprague-Dawley rats were each implanted with one smooth shelled 20 ml tissue expander filled with normal saline. At intervals of six, seven, eight, nine and 12 weeks post-implantation, animals were sacrificed and the saline within the expanders was analyzed by potentiometric ion analysis for the concentrations of sodium, chloride, potassium, and bicarbonate; the osmolarities of the solutions were also determined. A similar analysis was performed for three control samples of normal saline. The results are shown below:

A linear correlation analysis of the individual electrolyte concentrations in each expander was performed to determine their relationship with time of implantation. No statistically significant correlation was observed at the p = .05 level of significance (two-tailed).

In light of the absence of a temporal relationship, the mean electrolyte concentrations among all the expanders were compared against the mean electrolyte concentrations in the controls using a Cochran t-test. The t-test indicated no statistically significant differences between the mean sodium and chloride concentrations from the expanders and those of the controls at the p = .05 level. The differences between the mean potassium and bicarbonate

|                     | mean [Na⁺] (mEq/l) | mean [K⁺] (mEq/l) | mean [Cl⁻] (mEq/l) | mean [HCO₃⁻] (mEq/l) | mean Osmolarity |
|---------------------|--------------------|-------------------|--------------------|----------------------|-----------------|
| Control saline      | 147.80             | 0.00350           | 181.68             | 0.19                 | 327.48          |
| SEM                 | 0.67               | 0.00002           | 1.50               | 0.02                 | 1.64            |
| Expander saline     | 145.55             | 0.0116            | 180.10             | 0.51                 | 325.6           |
| SEM                 | 0.33               | 0.00010           | 2.11               | 0.01                 | 2.44            |
concentrations from the expanders and controls were found to be statistically significant at the \( p = .05 \) level. These differences, however, could be accounted for by contamination of the expander saline with interstitial fluid during inflation. We conclude that no significant changes in the average concentrations of the major electrolytes of saline within tissue expanders were observed \textit{in vivo}.

\textbf{An Analysis of Organization and Utilization of Medical Care During the 1995 Special Olympics World Games.} Lillian Anne Oshva. Yale University School of Medicine, New Haven, Connecticut.

The Special Olympics World Games (SOWG) were held in New Haven, Connecticut on July 1 to 9, 1995. The 1995 World Games differed from previous versions by the number of participants involved (ten thousand), and by the choice of a relatively small host city. One of the challenges of the 1995 World Games was to design and implement a comprehensive healthcare system for participants in order to avoid overloading local medical resources. The goal of this study is to test the hypothesis that the SOWG healthcare system succeeded in minimizing the impact of the World Games on local medical resources.

The study describes the population covered by the SOWG healthcare system, analyzes the utilization of that system by SOWG participants, and compares utilization of Yale-New Haven Hospital (YNHH), the official hospital of the SOWG, before, during, and after the SOWG and during the same time period the previous year.

If the YNHH Emergency Department had been used as the primary healthcare provider for participants, similar to the plans of previous SOWG, Emergency Department Visits during the week of July 2 would have risen by 136 percent to 2,806. Between June 28 and July 10, the average number of visits to SOWG medical sites in the field (dormitories, field sites and Smilow combined) was 107 for a total of 1,395 visits over the entire period. Average daily referrals to YNHH during this same time period was 10 with a total of 150 total visits at all times by SOWG participants. There were more injuries (54 percent) in the field than illnesses (41 percent), but of all transfers to YNHH for further evaluation, 74 percent were for illness and only 26 percent were for injury.

The data suggest that the SOWG healthcare plan adequately addressed the medical needs of official participants and successfully buffered the impact of the Games on local healthcare resources, and that it should be considered as a model for future World Games.

\textbf{The Accuracy of Magnetic Resonance Imaging in Determining the Volume of the Prostate Gland.} Robert J. Ostfeld. Departments of Diagnostic Radiology and Urology, Yale University School of Medicine, New Haven, Connecticut

The purpose of this study was to evaluate whether magnetic resonance imaging (MRI) could accurately measure the volume of the prostate gland. Six patients were consecutively and prospectively evaluated. The mean age was 63 \( \pm 6 \) years. All patients had biopsy proven adenocarcinoma of the prostate and were scheduled for radical prostatectomy prior to entrance into the study. All patients had MRI of the prostate within one week prior to their surgery. The volume of the prostate was estimated from the MRI image. Each MRI prostate volume was then compared to the corresponding prostate volume determined from the surgical specimen. The surgical volume was considered the "gold standard." The radiologists and surgeons were blinded to the results until completion of the study. The mean difference between the two volume measures was 16.98 \( \pm 20.98 \) cm\(^3\). The larger volumes were found more frequently in the surgical group. The volumes of the two
The Haven, Barbara A. Ward.

Evaluation underway breastidentified of toward quantified, nant may proceed directly ratio of MRI were at least 20 cm³ smaller than their corresponding pathological volumes. The other three corresponding volumes were within 6 cm³ of each other. Given the small sample size and the high variance of the volumes, we recommend that subject inclusion continue particularly in light of the modest positive correlation.

The Prospective Role of Duplex Ultrasound and Magnetic Resonance Imaging in the Evaluation of Axillary Lymph Node Involvement in Breast Cancer. Jill Suzanne Oxley, Kenneth J.W. Taylor, Robert C. Smith, William B. Stewart, Darryl Carter and Barbara A. Ward. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

In this investigation, we hypothesized that evaluation of axillary lymph nodes with ultrasound examination, using both morphologic and flow characteristics and/or evaluation with gadolinium-enhanced magnetic resonance imaging (MRI) may detect metastatic involvement of the axillary lymph nodes in breast cancer patients.

We first set out to establish a protocol for gadolinium-enhanced MRI of the axilla using cadaveric dissection and MRI, and MRI of a normal subject. Subsequently, patients with biopsy-proven breast cancer were recruited to undergo axillary ultrasound and/or MRI examinations prior to axillary lymph node dissection. Six patients underwent axillary ultrasound examinations; one patient underwent MRI as well. The results of the exams were compared with the surgical pathology; in addition, a retrospective analysis of the identified lymph nodes was performed in order to identify specific characteristics that may prove useful in distinguishing benign from malignant axillary lymph nodes.

Of the six patients studied, ultrasound examination correctly classified two of three histologically positive axillae and one of three histologically negative axillae. Both ultrasound and MRI failed to detect metastatic involvement in one patient. Retrospective analysis of the lymph nodes identified with ultrasound suggests that the length-to-width ratio (L/W ratio) of the nodes may correlate with tumor involvement; lymph nodes with a L/W ratio of more than 1.5 were more likely to be normal, while lymph nodes with a L/W ratio of less than 1.5 were more likely to be metastatic. Other characteristics that were not quantified, such as abnormal flow patterns and loss of central echogenicity, may also point toward tumor involvement in axillary nodes.

In conclusion, as a direct result of this preliminary work, an ongoing investigation is underway using ultrasound in the evaluation of axillary lymph nodes in patients with breast cancer. While the accuracy is limited to date, further refinement of this technique may identify which characteristics are most valuable in differentiating benign from malignant lymph nodes. Patients with normal-appearing nodes would still need to undergo axillary lymph node dissection in order to rule out micrometastases, but patients with abnormal-appearing nodes, especially in the context of high clinical suspicion, could potentially proceed directly to adjuvant therapy. In addition, although not investigated here, the use of ultrasound-guided fine needle aspiration biopsy when abnormal appearing nodes are identified may further refine this technique.
The Effects of Hypoxia on the Expression of VEGF in Muller Cells of Rat Retina. Joe A. Pastrano (Sponsored by Colin J. Barnstable). Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut.

Introduction: Vascular endothelial growth factor (VEGF) and its two known receptors have been implicated in angiogenesis during embryo development and neovascularization in certain pathological states such as in tumorigenesis and diabetic retinopathy, in which vessel formation invades the retinal disc. Animal studies, in this latter case, have shown that ischemia is associated with neovascularization and retinopathy with a concomitant increase in retinal VEGF expression. What cells in the retina are responsible for sensing O2 concentrations and expressing VEGF have yet to be determined.

Hypothesis: We hypothesize that the Muller cell, a glial cell spanning the whole thickness of the retina, may play a role in sensing changes in O2 tension, resulting in an increase in VEGF and perhaps VEGF receptor, expression.

Methods: Muller cell lines were established from rat retina and grown in monolayer. One group was grown in 10 percent CO2 and balanced air, while the other group was grown for 48 hrs in three percent O2, five percent CO2, with N2 infusion. The cells of both were homogenized and their mRNAs extracted. They were analyzed by PCR methods, using pre-constructed primers, for the expression of VEGF and its receptors (Flt-1 and KDR). These tests were further corroborated with Southern hybridization of the PCR products, using radiolabeled probes for each of the aforementioned gene products. C6 glioma cells, in which VEGF is known to be expressed, were used as a control.

Results: VEGF is expressed in Muller cells growing in normal oxygen tension (21 percent), as confirmed by both PCR and Southern hybridization. Of the two receptors, only Flt-1 was found to be expressed in Muller cells. KDR was not expressed in Muller cells grown in normoxic conditions, although KDR was found to be expressed in whole retina. Muller cells grown in hypoxic conditions (3 percent O2) had a four-fold increase in VEGF expression compared qualitatively with those grown in normoxic conditions. Further, these Muller cells grown in hypoxia showed a several-fold increase in the expression of Flt-1.

Conclusion: Previous studies have suggested an increased expression of VEGF in the retina under ischemic conditions. From our studies, it is clear that the Muller cell within the retinal layers plays a significant role in sensing hypoxia and responding with increasing expression of VEGF and of the VEGF receptor, Flt-1.

Grading the Report Card: An Investigation of Consumer-Directed Information Disclosure as the Basis of Quality Assurance Systems in Health Care Reform. Jason Ross Penzer (Sponsored by Angela Holder). Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

This thesis investigates the hypothesis that consumer-directed quality information dissemination, in the form of health care report cards, is practically, technically and theoretically inappropriate as the foundation of quality assurance systems in health care reform. The investigator examines the emergence of consumer-directed information disclosure proposals in the public health care reform debate and in the private sector. By drawing on the literatures of cognitive psychology, marketing, existing statutory information disclosure and case studies of current report card efforts, the author reveals the pitfalls
of relying on health care report cards as a quality assurance system. The research supports the hypothesis, and the investigator concludes that despite their rising popularity in legislative reform and the private sector, report cards cannot currently assure quality, given limitations in the state of the art of quality measurement and ignorance about the ways in which consumers would process disclosed information.

**Modulation of Experimental Autoimmune Encephalomyelitis by Gene Transfer of the Self Antigen Myelin Basic Protein into the Bone Marrow of Mice.** Timothy R. Peters, David M. Bodine, Kevin T. McDonagh, Henry F. McFarland, Dale E. McFarlin, Neal S. Young, Arthur W. Nienhuis and Michael K. Racke (Sponsored by Charles A. Janeway, Jr.). Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland.

Expression of a foreign major histocompatibility complex (MHC) molecule introduced into bone marrow stem cells of mice by retrovirus-mediated gene transfer results in their expression on the surface of thymic cells. These cells recognize the foreign MHC as self, deleting reactive T-cells, as was demonstrated by prolonged survival of MHC-specific skin grafts. We asked whether tolerance can be induced to a self antigen (as opposed to a foreign antigen) using a similar gene transfer strategy.

Experimental autoimmune encephalomyelitis (EAE) is a model for T-cell mediated autoimmunity in which mice develop a CD4 T-cell response against myelin basic protein (MBP). EAE results in CNS demyelination and ascending paralysis. We successfully created high titer ecotropic retrovirus producer cell lines that contain cDNA encoding the 21.5 kD MBP isoform and show diffuse cytoplasmic immunofluorescent staining by antibody specific for MBP. 3T3 fibroblasts infected with recombinant retrovirus contain unarranged viral sequences, and infected mouse hematopoetic cells show proviral expression. We have reconstituted EAE susceptible mice with bone marrow cells infected with recombinant retrovirus and demonstrated persistence and transcription of provirus in the peripheral blood cells and thymus of these mice.

We have induced EAE in over 70 B10.PL(73NS) mice transplanted with marrow transduced with MBP-containing retrovirus or control retrovirus. Our experiments have shown no decrease in EAE susceptibility in MBP-transduced mice, and our results suggest that these mice may contract more severe disease than control mice. Further, we have observed in preliminary studies that EAE resistant C57BL/10 mice transplanted with MBP-transduced marrow show susceptibility to EAE, unlike control mice. We conclude that expression of the autoantigen MBP in hematopoetic cells may confer susceptibility to EAE.

**Prostaglandin E₂ Alters Transforming Growth Factor-β Binding and Function in Fetal Rat Bone Cell Cultures by Cyclic-AMP Dependent Effects.** Tony H. Pham, Sandra Casinghino, Thomas L. McCarthy, and Michael Centrella. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

One response by bone cells to injury is expression of prostaglandin E₂ (PGE₂), which rapidly induces cyclic-AMP (cAMP) synthesis. In contrast to the effect of transforming growth factor β (TGF-β), another local bone regulator, PGE₂ does not directly increase DNA or collagen synthesis in osteoblast-enriched cultures. These studies examined the effect of PGE₂ on TGF-β binding and function in bone cell cultures. Confluent osteoblast-enriched cultures prepared from fetal rat bone were examined for changes in DNA and
protein synthesis by metabolic labeling. TGF-β receptors were examined by 125I-ligand binding and gel electrophoresis, and messenger RNA levels by northern analysis. Cyclic-AMP-dependent effects were evaluated in cultures transfected to over-express the catalytic subunit of protein kinase A (PKA). Pre-exposure to PGE2 significantly reduced the stimulatory effect of TGF-β on DNA synthesis by 43 ± 6 percent. Up to a 53 ± 4 percent decrease in the stimulatory effect of TGF-β on collagen synthesis was observed with PGE2 pretreatment. In addition, PGE2 transiently increased betaglycan expression, a proteoglycan that accumulates TGF-β but does not directly mediate its activity. However, it had no significant effect on TGF-β binding to type I and II TGF-β receptors that transduce biochemical signals. Treatment with PGE1 and forskolin, which also induce cAMP expression, produced comparable results, while PGF2a was ineffective in both regards. Finally, betaglycan expression was increased in cultures transfected to overexpress the catalytic subunit of PKA. Our results therefore demonstrate that PGE2 opposes the effects of TGF-β on bone cell activity and suggest that it increases betaglycan expression by a protein kinase A-dependent event. This binding site redistribution may, at submaximal TGF-β concentrations, diminish the proportion of ligand that can associate with signal transducing type I and II receptors. Accordingly, PGE2 induced in bone following trauma could regulate specific phases of TGF-β-mediated remodeling or repair.

**Varicella Pneumonia in Pregnancy.** Ioannis E. Platis, Oscar Einarsson, Hilder Hardardottir and Lynn T. Tanoue. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, and Department of Obstetrics and Gynecology, University of Connecticut School of Medicine, Farmington, Connecticut.

Maternal varicella pneumonia is historically associated with considerable mortality. Although the disease is uncommon, death is reported in up to 40 percent of patients. The purpose of this investigation is to review the experience with varicella pneumonia in pregnancy at our institutions, to evaluate the severity of respiratory complications in pregnant women with varicella pneumonia, and to assess current mortality.

Cases were identified by computer search of medical records at Yale-New Haven Hospital and New Britain General Hospital between 1985 and 1995. We describe 12 patients with varicella pneumonia diagnosed during pregnancy. Median gestational age was 25.5 weeks (range 17 to 40 weeks). Eight patients (67 percent) were hispanic. Seven of these patients were from outside the United States. Median P02/PaO2 was 0.16 (range 0.09 to 0.80) in 10 patients in whom arterial blood gases were measured. Six patients (50 percent) developed respiratory failure requiring mechanical ventilation. In the 6 patients requiring mechanical ventilation, median PEEP administered was 11 cm H2O (range 7.5 to 15 cm H2O). All patients were treated with acyclovir. There were no maternal deaths. Ten fetuses are known to have survived; none exhibited evidence of congenital varicella infection.

These data confirm that maternal varicella infection may cause severe respiratory morbidity but suggest that maternal mortality has substantially decreased. Although mortality was not evaluated prospectively and randomly, the data strongly suggest that the introduction of acyclovir in the management of varicella pneumonia in pregnancy is the main reason for the observed decrease in mortality.
Effects of Cryopreservation and Deconstruction on the Dermal Glycosaminoglycan Content of Human Skin. Matt Poggi, Giselle Chapo, Matt Klein and Charles B. Cuono. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut

This study compares the glycosaminoglycan (GAG) composition of fresh, cryopreserved and deconstructed human dermis (DhD), i.e., native skin soaked in an excess of PBS for 40 days at 37°C. We also determine the kinetics of GAG leaching during the standard preparation of DhD.

Samples of fresh skin and cryopreserved skin were obtained from the Yale Skin Bank which follows American Association of Tissue Banks practices. DhD was prepared by soaking cadaveric skin in an excess volume of PBS with a combination of penicillin and streptomycin and of amphotericin B for 40 days at 37°C. PBS was changed every 5 days until incubation was completed. For determining the kinetics of GAG leaching, aliquots of PBS were removed from DhD cups at intervals of 1, 2, 4, 7, 21, 28, 35 and 40 days. GAGs were isolated from tissue specimens using a standard protocol. The GAG isolates and aliquots of DhD PBS were subject to quantitative and qualitative analysis with two independent colorimetric assays, Stains-all® and DMMB, and electrophoresis followed by scanning densitometry.

Based on both colorimetric assays and supported by electrophoretic separation followed by scanning densitometry, no significant difference was found between the dermal GAG content in fresh or cryopreserved skin. We also determined that GAG leaching from skin stored in aqueous crystalloid solution prior to, cryopreservation begins immediately and is maximum within 2 to 7 days. We determined that DhD is exhausted of GAGs at its completion. This finding supports our contention that DhD is closer to a deconstructed dermal construct than merely "de-epidermized," as it was erroneously dubbed in the literature. We also determined that the Stains-all® and DMMB assays are comparable. There was no statistically significant difference in values obtained with either assay.

Microcalcifications in Benign and Malignant Breast Disease. Sarah H. Poggi, H. Catherine Skinner and Darryl S. Carter, Department of Geology and Geophysics, Yale University and Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

This study histologically characterized 155 sequential breast biopsy or mastectomy specimens with microcalcifications. On selected samples scanning electron microscopy (SEM) was used to semi-quantitatively analyze the mineral portions utilizing the JEOL JXA 8600 Superprobe installed at the Yale Geology Department. There was an overall incidence of microcalcification of 27 percent, which included 24 percent of benign cases and 39 percent of malignant cases. Predominantly calcium phosphates were present, though calcium oxalate represented 3 percent of the microcalcifications present. Calcium phosphates were found in collagenous stroma (31 percent of total) and in ducts (69 percent of total). Small (10 µm) ductal calcifications were likely to be found in sclerosing adenosis, fibrocystic changes, ductal hyperplasia and DCIS. Stromal calcifications were found most commonly in patients with fibrous proliferation, including those older patients with fibroadenomas, scar from biopsy or implant contracture, radiation therapy or sclirrrous reaction from invasive ductal carcinoma. Lobular lesions were generally not calcified. Those calcium phosphates found in the stroma of a rapidly growing invasive ductal cancer and recent surgical scar had a calcium to phosphorous weigh percent ratio of less than 2.15, suggesting the presence of hydroxyapatite and an additional calcium phosphate phase. The slightly higher ratio (2.17) found in the fibroadenoma suggested that this
was a more mature apatitic system. The calcium phosphates found in the necrotic ducts of comdeo DCIS specimens and in the benign ducts of sclerosing adenosis had calcium to phosphorous ratios significantly above 2.15, suggesting the presence of hydroxyapatite and a second, non-phosphate, phase. These findings argue for a different microenvironment for precipitation of these two mineral systems, with those associated with ducts having more available calcium. Also of interest was the presence of fluorine at weight percent consistently above one percent, much greater than that found in the hydroxyapatite mineral portion of bone or teeth, which is around 0.02 percent.

**The Utility of Adjuvant PUVA Therapy and Prognostic Significance of Extent of Skin Involvement in CTCL Patients Following Total Skin Electron Beam Therapy.** Peter Quiros (Sponsored Barry M. Kacinski and Lynn D. Wilson). Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut.

Background: To evaluate the utility of adjuvant oral psoralen and ultraviolet-A light (PUVA) therapy in the clinical management of stage T1 and T2 cutaneous T-cell lymphoma (CTCL) patients treated with total skin electron beam therapy (TSEBT) and to define the prognostic significance of the extent of skin involvement (ESI) with respect to disease-free (DFS) and overall survival (OS) in CTCL patients with T3 disease following TSEBT.

Methods: Between 1974 and 1993, TSEBT (36 GY, 1 GY/day for 9 wks, 6 MeV electrons) was administered to a total of 213 patients. One hundred fourteen of these were classified as either T1 or T2 early-stage CTCL patients, and 46 were classified as T3 (Tumor) stage CTCL. In 1988, we began to offer T1 and T2 patients adjuvant PUVA therapy, within two months of TSEBT. We compared the effect of PUVA therapy on the DFS and OS of this group of patients to the T1 and T2 patients who received identical TSEBT but without adjuvant PUVA prior to 1988. The T3 stage patients' records were retrospectively evaluated, and the percentage of total skin surface involved was calculated. These patients were analyzed with respect to DFS and OS. The median follow-up for the T1 and T2 stage patients was 62 months (3.4 to180), while the median follow-up for the T3 stage patients was 37.5 months (1.6 to 93).

Results: The majority of patients achieved a complete clinical remission (CCR) by the completion of TSEBT (97 percent of T1, 87 percent of T2, and 78 percent of T3). Overall, 40 percent of the T1 and T2 patients relapsed in the skin after TSEBT. But when analyzed by post-TSEBT therapy, 14 percent of those who received adjuvant PUVA relapsed, compared to 49 percent of those who received no adjuvant PUVA therapy in the same time period. The median DFS for the two groups were 103 months and 50 months respectively (p < .018). The overall survival was not significantly altered.

DFS for the T3 patients was 12 percent at 36 months with approximately 28 percent OS. However, when stratified by ESI, those with 9 percent or less ESI had a median DFS of 63 months, whereas those with 10 percent or greater ESI had a median DFS of only four months (p < .045). The median OS for each group was 65 months and 24 months respectively (p < .040).

Conclusions: Adjuvant PUVA after TSEBT appears to be a safe effective means of preventing cutaneous relapse in CTCL patients with T1 or T2 disease. Furthermore, ESI in T3 stage patients is a valuable prognostic indicator of DFS and OS for those treated definitively with TSEBT.
Impairment of Flow and Functional Reserve in Myocardium Remote to a Critical Coronary Stenosis in the Presence of Dobutamine Stress. Brian D. Rinker, Don Dione, Paul DeMan, Eliot Heller, Edouard Daher, Frans J.Th. Wackers, and Albert J. Sinusas. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Ischemic heart disease is the leading cause of mortality in the Western World. Successful management relies critically on the ability to make an early diagnosis, and in recent decades, myocardial scintigraphy in conjunction with pharmacologic stress has been an indispensable diagnostic tool. However, a potential limitation lies in the fact that a coronary stenosis may affect flow reserve in remote myocardium, thus limiting the stress agent's ability to produce flow heterogeneity. This impairment in remote zone flow reserve may be due to the changes in ventricular strain pattern which accompany a single-vessel stenosis. The present study was undertaken to determine whether there is impairment of flow reserve in remote myocardium following a coronary stenosis in the presence of dobutamine stress, and if that impairment is associated with changes in remote zone radial strain.

The study was undertaken on three groups of anesthetized, open-chested dogs: Group 1 (control) dogs without a stenosis, Group 2 dogs with a stenosis which ablated the hyperemic response but did not affect resting flow; and Group 3 dogs with pre-stenotic infusion of dobutamine and high fidelity micromanometers allowing precise segmentation of the cardiac cycle. Myocardial thickening fraction (percent TF) in the stenotic LAD and control LCX region was measured at baseline and during dobutamine stress with sonomicrometers. Flow was measured using radiolabeled microspheres. An increase in the amount of myocardial thickening occurring in isovolemic systole was used as the index of radial strain.

A marked impairment of post-stenotic normal zone flow reserve in response to high dose dobutamine was observed in Groups 2 and 3. This impairment in flow reserve was concomitant with an increase (although not statistically significant) in the percent of myocardial thickening occurring during the isovolemic phase of systole. These observations lend support to the proposition that changes in radial strain are responsible for the observed impairment of flow reserve distant to a "critical" coronary stenosis.

Evaluation of Prostate Specific Antigen and Other Prognostic Factors in Patients Treated with Postoperative Radiotherapy for Carcinoma of the Prostate. Theodore J. Robnett, Ronald P. Ennis and Lynn D. Wilson. Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut, and Department of Radiation Oncology, College of Physicians and Surgeons of Columbia University, New York, New York.

Selection criteria for prostate cancer patients who would benefit from postoperative radiotherapy (RT) are controversial. The combined institutional experience of the College of Physicians and Surgeons of Columbia University and the Yale University School of Medicine was reviewed to identify prognostic factors relating to biochemical failure.

Between January 1989 and June 1994, 48 prostate cancer patients received postoperative RT, with 43 patients being evaluated. The median age of the cohort was 65 (50 to 75) years. Twelve patients had pathologic T2 disease; 36 had pathologic T3 disease. Pre-RT PSA was detectable in 27 patients. Multi-field techniques which excluded the pelvis were employed. Biochemical failure was defined as occurring when PSA became detectable. Disease free survival (DFS) was evaluated by life table analysis. The Cox proportional
hazards model was used to evaluate significance of preoperative PSA, pre-RT PSA, margin status, capsular invasion, seminal vesicle involvement, Gleason score, RT dose, and surgery-RT interval. Patients were evaluated every 3-6 months. Median follow-up was 35 months.

The median Gleason score was 6, the median preoperative PSA was 13.6 (3-100), and the median pre-RT PSA was 0.7 (< 0.1-74). Median surgery-RT interval was 11.5 (1.5 to 75) months. There was no significant difference in biochemical DFS between institutions. Thirty-three of 48 patients (68 percent) were biochemically free of disease within six months after RT, with 12 of 33 relapsing. One, three, and five year actuarial biochemical DFS rates were 65 percent, 51 percent and 29 percent respectively. Multivariate analysis revealed preoperative PSA to be associated with biochemical DFS (p < .001, N = 38). When multivariate analysis was performed excluding preoperative PSA (N = 43), pre-RT PSA (p < .01) and Gleason score (p < .001) were significant. A Pre-RT PSA "cutoff" of 0.3 was associated with biochemical freedom from disease (p < .001).

Preoperative PSA is significantly associated with biochemical DFS. Excluding this variable, pre-RT PSA and Gleason score were prognostically significant. Patients considered for postoperative RT appear to benefit from treatment prior to, or at the time of detectable rise in PSA. Lead time bias may play a role in these results.

Acute Vasoactive Effects of Estradiol on Cerebral Vasospasm in Women with Subarachnoid Hemorrhage. Joshua M. Rosenow, Phillip M. Sarrel, Issam A. Awad, Lawrence M. Brass. Departments of Surgery, Neurology, and Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

Approximately one-third of patients diagnosed with subarachnoid hemorrhage (SAH) will develop symptomatic vasospasm leading to delayed ischemic deficits. Estrogen has been shown to either vasodilate or inhibit vasoconstriction in several vascular beds. This may occur by either a calcium channel-blocking effect or via induction of nitric oxide release by vascular endothelium.

We studied nine women diagnosed with aneurysmal subarachnoid hemorrhage. Estradiol–17β (E2) and progesterone levels were measured daily. Women received daily monitoring for vasospasm with transcranial Doppler (TCD) ultrasound. Patients whose mean blood flow velocities (BFV) exceeded 120 cm/sec were deemed to be in vasospasm and received E2 [1 mg SL (Estrace®)]. TCD monitoring was conducted for 20 min prior to E2 administration and for 120 min post-administration. E2 levels were measured prior to and two hours after administration of E2.

Three women were given estradiol a total of four times early in their hospitalization. Mean BFV showed a statistically significant decrease from baseline beginning at 25 min (p < .02) and continuing through the 120 min monitoring period (p < .005 at 120 min). Mean BFV were not significantly decreased in those trials conducted greater than 6 days post-hemorrhage (N = 2). There was no significant change in either peak flow velocity or pulsatility index.

This preliminary study demonstrates that estradiol–17β administration leads to a significant decrease in the mean BFV of women who have suffered a SAH. The effect of E2 is greater and begins sooner when it is given prior to 6 days post-hemorrhage. These early data warrant the further investigation of this finding, including double-blind studies to determine the efficacy of E2 supplementation in preventing vasospasm.
Continuous Monitoring and Display of Emergency Department Patient Flow and Waiting Times: A Method to Reduce Overall Length of Stay. Martin Rossip (Sponsored by Richard Nierenberg). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut

Insofar as long waiting times and overcrowding in urban emergency departments contribute to inaccessibility of health care for a large and vulnerable population, this study attempts to determine whether it is possible to reduce patient waiting times and length of stay in the emergency department through the use of a patient tracking system which provides real-time display of patient status and process durations. Software based on continuous quality improvement techniques was used as a data collection and display tool to test this hypothesis.

The study population consisted of all patients seen in one section of the emergency department (ED) at Yale-New Haven Hospital during the study period, which was divided into three equal time segments. Total length-of-stay for 467 patients were prospectively tracked for eighteen days. The control group consisted of those patients (N = 169) seen during the first six day segment, with patient information and triage time displayed using a conventional dry-erase marking board. The intervention group consisted of those patients (N = 150) seen during the second six-day segment, during which a computer monitor was conspicuously placed adjacent to the existing dry-erase board. The monitor displayed the same columns as the dry erase board, but added two items: the current status of each patient and the time spent waiting within that status. In addition, the software automatically alerted staff when waiting time exceeded a predetermined interval by highlighting the patient’s status in red. During the third six-day segment, the monitor was removed and a second control group (N = 148) was tracked, again using only the standard dry-erase board. Waiting times and overall length of stay were compared for each study period.

Average length of stay for segments one, two and three were 270.3, 240.7, and 280.6 minutes respectively. Length of stay decreased during the intervention period by 10.95 percent (p < .05). Following removal of the intervention, length of stay increased by 16.58 percent (p < .05). Overall, the weighted mean reduction of length of stay associated with the intervention was 12.5 percent or 34.4 minutes.

The study supports the conclusion that real-time display and monitoring of patient status and waiting times decreases overall ED length of stay. The hypotheses that heightened caregiver awareness of patient waiting times alters caregiver task management habits resulting in a reduction of overall time spent in the ED was also supported by the study.

Children Before the Court: Characterization of Juvenile Delinquents in Urban America. Emily J. Roth (Sponsored by Donald J. Cohen). Child Study Center, Yale University School of Medicine, New Haven, Connecticut.

In this study, a sociodemographic profile of arraigned and sentenced juveniles at the New Haven Detention Center was compared to a matched cohort of New Haven school children. The data revealed significant sociodemographic differences between the juvenile offender population and the compared school population, suggesting early predictors to juvenile delinquency. These predictors may not cause delinquency but represent early signals that a child is at risk. More specifically, the results showed that the arraigned juvenile court population (n = 89) had significantly more males (p = 0.00), African-Americans and Hispanics (p = .00) and children with a history of previous offense (p = .00) as compared to the school population. Likewise, the sentenced juvenile court population compared to the school cohort had more males (p = .00) and more African-Americans and Hispanics (p = .01). In addition, the sentenced cohort had a significantly higher truancy rate (p = .01),
suspension rate (p = .00), and were substantially more likely to have been previously seen as victims of abuse or neglect at DCYS (p = .00). Finally, juveniles from the sentenced population lived disproportionately in single-parent homes (p = .00) with larger households (p = .00), and were more likely to be receiving public assistance (p = .02). Differences in history of parental alcohol abuse were not significant (p = .09).

Mental health workers should educate teachers, parents, and police about predictors of delinquency in their communities so that troubled youth may be better identified, assessed and referred to appropriate preventive programs early on.

Furthermore, mental health professionals should work within the court system to evaluate juvenile offenders so as to better direct their rehabilitation. The data support the possibility for early identification of risk factors for delinquency and the need for intervention that addresses these risks.

**Tic Disorders and the Pediatrician: Current Understanding and Approach to a Model Neuropsychiatric Condition.** Jacob Walton Roth (Sponsored by Donald J. Cohen). Child Study Center, Yale University School of Medicine, New Haven, Connecticut.

Tic disorders represent a spectrum of transient and chronic conditions with substantial prevalence among children and adolescents and manifesting with a range of disruptive pathology. The present study was initiated to establish the level of knowledge and comfort among pediatricians with regard to tic disorders and associated behavioral symptoms, while further gauging their approach to managing such patients. A questionnaire was constructed to achieve these aims and sent out to a randomized sample of 300 Connecticut pediatricians.

The resulting data revealed a dramatic lack of comfort and familiarity among pediatricians with the diagnosis, natural history and approach to treatment of tic disorders as well as their common comorbid behavioral conditions. The participants further painted an exceedingly bleak view of their residency training in both general behavioral issues as well as tic disorders. Importantly, though, the minority who rated their residency training as good in these realms, revealed significantly greater numbers considering themselves able in the care of such patients. Although years of practice experience and extent of outpatient exposure also bore statistically significant positive effect on many variables, the absolute improvement remained slight, demonstrating that simple exposure serves as no substitute for the inclusion of at least reasonable teaching in behavioral issues and tic disorders during residency.

These findings suggest that many clinicians likely continue to underdiagnose tic-symptomatology and its associated behavioral symptoms, as well as lack the knowledge and ability to effectively treat such patients even when the pathology is recognized. This seems particularly concerning in light of the considerable prevalence of these disorders and enduring impact insufficient intervention and care may have on the child's social development, sense of self-concept and overall functioning. Indeed, this investigation lends credibility to those advocating improved developmental/behavioral training in pediatric residency as well as underscores specifically the need for more education within the pediatric community with regard to tic behaviors and the full spectrum of their symptomatology.
Reconsidering Trust in the Physician-Patient Relationship. Julie Anne Rothstein
(Sponsored by Robert J. Levine). Department of Internal Medicine, Yale University
School of Medicine, New Haven, Connecticut.

Since Hippocratic times, trust has been considered a fundamental aspect of the physi-
cian-patient relationship. During recent years, the trust between doctor and patient has
deteriorated. Many factors have contributed to this development, including the cultural
environment, technology, and changes in health care delivery. One might say that despite
the importance of the notion of trust to medicine, the actual meaning and limits of trust
are poorly defined. Because of these factors, one writer has claimed that in today’s world,
trust between physician and patient is an “incoherent” concept. The purpose of this thesis
is to reexamine trust within the physician-patient relationship and determine whether the
notion of trust is an ideal to be preserved, disposed of as incoherent or refined according
to realistic standards and expectations at the end of the twenty-first century.

To explore the applicability of trust to the physician-patient relationship, this thesis
will examine the notion of trust generally, considering the many ways and levels in which
trust functions in our everyday lives. Through this analysis, certain principles and aspects
of trust will emerge that will help to clarify the concept. Also, factors that allow trust to
flourish will be explored. A case study will be used to show how the refined notion of trust
functions in the physician-patient relationship.

Instead of holding onto a romanticized and imprecise notion of trust or dismissing
the concept as incoherent, this thesis will show that trust is a crucial and irreducible aspect
of the physician-patient relationship. In order for it to function maximally in today’s med-
ical world, a clearer and more realistic notion of trust should be embraced. Recommendations for enhancing the environment for trust are provided.

An Evaluation of the Adequacy of Medical School Education on Neurological
Emergencies. Jay J. Schindler, Peter L. Meyor, and Issam A. Awad. Department of
Surgery, Yale University School of Medicine, New Haven, Connecticut

Over the past three decades, a great deal of energy has been devoted to training med-
ical personnel regarding life-threatening cardiovascular emergencies known generally as
“heart attacks.” This emphasis has not only produced medical professionals who are high-
lly qualified and well equipped to emergently diagnose and treat life-threatening cardio-
vascular emergencies but has created a community-wide awareness regarding these emer-
gencies and how proper intervention is of unquestioned benefit.

Although the sequelae of neurological emergencies, or “brain attacks,” are just as
devastating, it has been argued that there has not been the same emphasis devoted to diag-
nosing and treating acute neurological emergencies that are amenable to early interven-
tion. In order to assess the educational emphasis devoted to training prospective physi-
cians regarding the diagnosis and treatment of “brain attacks,” all US medical school
deans were surveyed to determine what is offered in their curriculum regarding neurolo-
gical emergencies. The same information was obtained for cardiovascular emergencies and
was used as the control. The results reflect a glaring lack of uniformity in the curricula of
U.S. medical schools to train medical students regarding the proper diagnosis and prompt
care of patients suffering from acute neurological events. Also, cardiovascular emergen-
cies are taught more than neurological emergencies by 4.22 hours of didactic sessions and
by 1.74 weeks of clinical rotations. This is in addition to specialty courses, offered or
required by 71.4 percent of the US medical schools, which emphasize cardiovascular emergencies.

Additionally, two expert panels were established to create questions regarding neurological emergencies with cardiovascular emergencies as the control. This questionnaire was distributed to interns at Yale-New Haven Hospital who responded correctly to 73.5 percent of questions regarding neurological emergencies compared with 86.9 percent of questions for cardiovascular emergencies. The results demonstrate a statistically significant difference in the ability of recent medical graduates to answer questions deemed basic to the management of neurologic emergencies as compared to cardiovascular emergencies.

Characterization of Two Endogenous Trophic Factors Involved in Regeneration of the Goldfish Optic Nerve. Jason M. Schwalb and Larry I. Benowitz (Sponsored by Susan Hockfield). Department of Neurosurgery, Children's Hospital (Boston), and Department of Surgery, Harvard Medical School, Boston, Massachusetts

Unlike mammals, lower vertebrates are able to regenerate injured pathways of the central nervous system throughout life. The factors responsible for initiating this process are unknown. We have shown that in dissociated cell culture, goldfish retinal ganglion cells regenerate their axons in response to two factors derived from goldfish optic nerve glia. Axogenesis factor 1 (AF-1) is a small, hydrophilic peptide (700 to 900 daltons) that is inactivated by treatment with proteinase-K, but heat-stable. A second factor, AF-2, is a basic polypeptide of about 12 kDa. Dissociated retinal cells remain viable in serum-free defined media for at least a week, but show little outgrowth. The addition of AF-1 induced up to 25 percent of cells in culture to extend processes > 75 μm in length by six days; AF-2 had a lesser but highly significant effect. Retrograde tracing studies confirmed that neurite outgrowth was from retinal ganglion cells per se. The effects of AF-1 and AF-2 were not secondary to enhanced viability, since neither overall cell survival nor the number of retinal ganglion cells was affected by the presence of these factors. The activity of AF-1 and AF-2 was not mimicked by several defined factors tested over a broad concentration range, e.g., NGF, BDNF, NT-3, NT-4/5, CNTF, αFGF, βFGF, taurine and retinoic acid. The concentration of AF-1 is considerably higher in conditioned medium than in optic nerve homogenates, suggesting that it is actively secreted; AF-2 has a similar concentration intra- and extra-cellularly. Insofar as AF-1 and AF-2 derive from cells of the optic nerve and act upon retinal ganglion cells, they are likely to be important in inducing optic nerve regeneration in vivo.

Death Due to Injury in Quito Ecuador. Eric S. Steenlage, David E. Chiriboga, Linda C. Degutis, Thomas H. Cogbill, Natalia Romero Sandoval and Stephen M. Cohn. Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Trauma is the leading cause of death in Quito, Ecuador (population 1.5 million). Our objective was to determine Quito's need for pre-hospital trauma transport systems. Two methods were used. The first was collection of transport times and outcome data from trauma victims with splenic injuries treated at two hospitals (one urban, one rural) in the United States over a two-year period. This information was compared to data from a similar population in Quito. The second method was examination of autopsy reports from trauma victims dying in Quito in 1994. Results from the splenic study were inconclusive. There were 69 splenic injuries with 14 deaths, none due to the splenic injury, at the urban U.S. hospital, and 39 splenic injuries with four deaths, one due to delayed presentation of the splenic injury, at the rural hospital. Surprisingly, there were only five patients with
documented splenic injuries at the Quito hospital during a six-year period. Analysis of autopsy data revealed an annual (1994) trauma death rate of 66.3/100,000 for Quito (U.S.: 37.0/100,000), with 64 percent sustaining head injuries, 30 percent thoracic injuries and 30 percent abdominal injuries. Only 28 percent of the trauma victims who died in Quito received medical attention before death. There were 39.1/100,000 motorvehicle-related deaths in Quito (U.S.: 18.8/100,000). Pedestrian injuries were the largest single category of trauma deaths in the city, representing 53 percent of all motor vehicle-related deaths (U.S.: 15 percent); there were 20.9/100,000 pedestrian deaths in Quito (U.S.: 2.7/100,000). Trauma is an excessive cause of mortality in Quito. Major factors contributing to this are the small percentage of trauma victims receiving medical attention prior to death and the high incidence of auto-pedestrian injuries. Injury prevention measures and adequate pre-hospital provider systems are desperately needed in Quito.

Disruption of the p53-Dependent G1 Arrest in Human Fibroblasts Results in Hyper-recombination. Lynne Meryl Strasfeld (Sponsored by M. S. Meyn). Departments of Genetics and Pediatrics, Yale University School of Medicine, New Haven, Connecticut

Genetic instability is a key feature of the cancer-prone inherited disease ataxia-telangiectasia (A-T). It has recently been demonstrated that one aspect of genetic instability in A-T is a marked elevation in the spontaneous rates of intrachromosomal mitotic recombination (Meyn, M.S., High spontaneous intrachromosomal recombination rates in ataxia-telangiectasia. Science 260:1327-1330). The damage surveillance network model proposed by Meyn attributes these high recombination rates to a lack of DNA damage-sensitive cell cycle checkpoints (Meyn, M.S. Ataxia-telangiectasia and cellular responses to DNA damage, Cancer Research 55:5991-6001, 1995). One prediction of this model is that disrupting p53 function in normal cells should increase their spontaneous rates of recombination by interfering with their p53-dependent G/S cell cycle checkpoint.

To test this prediction, we transfected SV40-transformed normal human fibroblast lines that each harbor a single integrated copy of a lacZ-based recombination vector (pLrec) with derivatives of a eukaryotic expression vector (pRep5) that contain either a dominant-negative p53 mutant (143val->ala), a wild-type p53, a human papilloma virus E6 gene (HPV18 E6), or a mutant E6 gene (HPV18 E6–OB). Expression of either the p53 mutant or the HPV18 E6 gene is reported to result in a loss of p53 function and loss of the G/S cell cycle checkpoint. Four independent p53143ala transformants of the control line showed an average 54-fold increase in spontaneous recombination rates when compared to their parent cell line. Elevations in spontaneous recombination rates were also detected following transfection with the HPV18 E6 gene. There was no significant increase in recombination rates following transfection with either the wild-type p53 or the HPV18 E6–OB gene.

In order to determine the etiology of the observed hyper-recombination, we employed FACS sorting to characterize cell cycle progression. Cell cycle analysis revealed an intact G/S checkpoint in SV40-transformed normal fibroblasts and the abrogation of this checkpoint in SV40-transformed A-T fibroblasts.

Our results support the hypothesis that the inactivation of p53 contributes to the hyper-recombination seen in A-T. Additionally, we have demonstrated supportive evidence for the existence of a G/S checkpoint in SV40-transformed human fibroblasts. Our findings add hyper-recombination to the spectrum of genomic instability caused by the loss of p53 function, a critical step in the development and progression of many tumors.
Breastfeeding the Preterm Infant: A Statistical Analysis of the Factors Affecting Choice and Ultimate Success. Lynn E. Sullivan, Richard A. Ehrenkranz, Barbara A. Ackerman, Jo Anne Metzger, Carol Bryce-Buchanan and Michael B. Bracken. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut

There is limited information about the factors that affect the mothers of preterm infants with respect to their choice to breastfeed and their success at breastfeeding. The purpose of this project was to interview women who delivered prematurely and examine the variables that influenced their decision of whether to breastfeed, and to define the factors related to their ultimate success. A feeding survey was administered by structured interview to 199 mothers of preterm infants cared for in the Yale-New Haven Hospital Newborn Special Care Unit, shortly after delivery; 109 women had selected breastfeeding, 85 had selected formula feeding, and five were undecided. A follow-up interview was administered to 73 women who were breastfeeding at the time of their infant's hospital discharge. The data were entered into an SAS database and were evaluated with a chi-square analysis and a stepwise logistic regression to determine which variables might affect feeding choice and successful lactation, and to estimate the effect that each of the significant variables independently had on the choice of feeding method. Eighty-three percent of women had chosen their feeding method prior to or during the first trimester; this finding is similar to the timing of feeding choice in the full-term population. The four variables having the greatest positive impact on a mother's final choice to breastfeed her preterm infant included the influence of friends and family who had previously breastfed an infant, if the mother did not smoke during her pregnancy, if the infant's father was the head of the household, and if breastfeeding was recommended by certain individuals. The analysis of the follow-up data revealed that the mean duration of lactation was 14.4 ± 1.0 weeks. The majority of women were successful as evidenced by the fact that 83 percent stated that they enjoyed breastfeeding and an overwhelming 94 percent stated that they would breastfeed another preterm infant.

Variability of Blood Lead Levels in Occupationally Exposed Construction Workers. Susan B. Truman, Petrie Rainey, Kathleen F. Maurer and Mark R. Cullen. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Construction workers who work on structures painted with lead paint are occupationally exposed to lead and risk lead toxicity. Surveillance programs such as the Connecticut Road Industry Surveillance Project (CRISP), monitor workers blood lead levels in order to identify exposed workers and to intervene. This study aims to identify the amount of variability in blood lead levels over time in order to find the optimal time interval for blood lead screening in occupationally exposed construction workers. Fourteen construction workers were recruited at three sites. Each subject kept a work log outlining tasks performed each day and also had his blood lead levels drawn three times per week for four to six weeks. In addition paint chip samples, air samples and wipe samples were obtained at the sites. Paint chips were 7.21 percent lead by weight. Air samples at one site measured from 190 to 12,580 µg/m³. Wipe samples found high lead levels on tarps and inside one respirator. There was very little variability in blood lead levels. With the variability due to analytic error parceled out, nine of the thirteen subjects for whom data was analyzed had blood lead levels that varied by less than 10 percent of the mean. Mean variability is 9.3 percent of the mean. No correlation was found between time and blood lead.
level, and no correlation was found between task performed prior to the blood draw and blood lead level. The environmental data support the conclusion that construction workers in Connecticut are exposed to lead and thus there is a need for surveillance programs such as CRISP. These data also suggest that construction workers have a gradual pattern of biological lead accumulation and blood lead levels that change slowly over time. Type of work done prior to a blood draw is not correlated with blood lead levels and is therefore unlikely to result in the identification of false positives in a screening program. These data support the conclusion that the screening interval of 30 days currently used by CRISP is adequate to identify exposed workers and is unlikely to miss any toxic exposures.

The Harvey Cushing Brain Tumor Registry: Changing Scientific and Philosophic Paradigms and the Study and Preservation of Archives. Christopher John Wahl (Sponsored by Dennis D. Spencer). Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Modern philosophies of the history of science dictate that the scientific community undergoes a depreciation of historical fact as part of the aftermath of paradigmatic shifts. In line with this Kuhnian model of scientific innovation, coupled inadvertently to historical devaluation, it appears that the hard data and physical products of scientific research are often lost, forgotten, or destroyed as the summations they provide are incorporated or excorporated from the scientific theories they tested. As theoretical constructs evolve or disintegrate, scientific history is rewritten. However, archives, collections, and databases of data which escape destruction are often of immense value to historians of science for reasons which lie completely outside the context of their initial creation. In point of fact, their value often depends precisely on a scientist’s unwitting inclusion of information which, in the environment of his research, he is unable to perceive or control. This paper examines three such collections. Harvey Cushing's Brain Tumor Registry is a document which chronicles the development of neurological surgery. It represents a remarkably complete diary of medicine and surgery in the early twentieth-century. Unexpectedly, the sublime photographic materials belonging to the Registry have inherent artistic value. The second collection represents the work of Samuel Morton and Louis Agassiz amassed to prove their pre-Darwinian hypothesis that all men evolved as separate species. Ironically, work on this racist theory produced daguerreotypes which survive today as the earliest surviving photographic images of African-Americans in this country—a valuable contribution to the black heritage. The final collection examined is the mid-twentieth-century work of William H. Sheldon, who struggled his entire career to link human morphology to psychology. His photographic archive, originally collected under somewhat inauspicious circumstances, was largely destroyed after a recent and shortsighted media exploitation. The paper condemns the senseless destruction of archival materials, especially when their value can not possibly be elucidated in the context of the present scientific and historic paradigm.

Accuracy of Antinuclear Antibody, Anti-Double Stranded DNA Antibody, and Anti-Histone Antibody Tests in the Setting of Anti-phospholipid Antibodies. David S. Wang and Linda K. Bockenstedt. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The anti-phospholipid syndrome is a recently recognized clinical entity manifested by disease related to sequelae of thrombosis. Anti-phospholipid antibodies associated with
clinical disease can occur as a primary syndrome or in association with autoimmune diseases, especially systemic lupus erythematosus (SLE). Because serologic laboratory tests are important in the diagnosis and management of patients with SLE, this research intended to assess the reactivity of anti-cardiolipin antibodies (ACA) with substrates in commercially available lupus-related serologic tests for antinuclear antibody (ANA), double-stranded DNA (dsDNA) and histone antibodies. Sera from the Yale Rheumatology Diagnostic Laboratory were selected that had previously been reported as ACA-positive. Sera were characterized for ACA by ELISA, ANA by HEp-2 cell immunofluorescence, anti-dsDNA antibodies by Crithidia immunofluorescence, and anti-histone antibodies by ELISA. ACA were adsorbed from ACA-positive sera which also tested positive for ANA, anti-dsDNA, or histone antibodies, after which sera were reassayed for these antibodies. After all serologic results were obtained, available charts were reviewed to correlate the serologic findings with the clinical presentation and diagnosis of patients whose sera had been analyzed. The results suggest that the presence of ACA in sera from patients without evidence of SLE can give rise to a false positive anti-dsDNA by Crithidia testing.

The Quest for Immortality: A Cross-Cultural, Multi-Discipline Perspective. Lisa Lynn Wei (Sponsored by William C. Summers). Section of History of Medicine, Yale University School of Medicine, New Haven, Connecticut.

The aim of this study is to demonstrate the parallel nature of the development, or evolution, of the quest for immortality in the Chinese and Western cultures from the religious, scientific, literary, cultural, and medical perspectives. The emphasis will be placed on the goals of each culture's search, the roles that religion, alchemy and medicine play, and the rich and colorful legends, beliefs, symbols, and language that blossomed around this search.

The similarities as well as the differences between the theorems and literature of immortality in China and in the West are extraordinary. And among the numerous aspects of each, I have chosen to focus on these five features: religion, alchemy, literature, art, and medicine — all interrelated — wherein the two systems display significant similarity and significant difference.

My thesis is generally based on a combination of primary research in the form of analyses of literary and historical texts and secondary research in the examination of studies that other researchers have made on the subject. I have drawn on a wide range of general and specialized reference works, focusing mainly on the published work of experts found in academic libraries (Yale University libraries, Texas Woman's University library, University of North Texas library). Primary sources include original and translated works of Chinese and Western literature, historical documents, religious texts and microforms. Secondary sources include books and journal articles on historical events and literary works, biographical sources, dictionaries, and encyclopedias. I have employed standard procedures and techniques in the writing of this paper: identifying, locating, and assessing primary sources, assimilating others' research, and then developing and expressing my own thoughts, feelings, opinions, and ideas.

In short, my hope is to assess the phenomena observed in the evolution of the Chinese quest for immortality as a direct parallel to that of Europe and the West, as a consequence of the universal human desire for eternal life, and in the light of its impact on later centuries and on the state of modern medicine. For it is truly remarkable and perhaps inevitable that throughout the history of humankind, nearly every culture must make its own journey in pursuit of the fulfillment of the inherent longing to live forever and that their journeys follow similar paths, transcending time and place.
There is indeed a correlation between the historical search for immortality and modern medicine’s continuation of that quest, be it using different methods and approaches. Not only is there a continuity between past, present, and future, but a continuity or convergence of the searches for immortality in all the cultures of the world. Whereas entire cultures may differ greatly, each culture’s journey in medicine and science in this field of research may cross and merge with those endeavors of other cultures sometime in the future, if they have not intersected and joined already.

**Molecular Analysis of Parathyroid Adenoma Pathogenesis.** H. Irene Wu (Sponsored by Andrew F. Stewart). Massachusetts General Hospital, Boston, Massachusetts, and Yale University School of Medicine, New Haven, Connecticut.

Genetic defects such as translocations or mutations are common mechanisms that are implicated in oncogenesis. The PRAD/cyclin D1 oncogene was discovered in a human parathyroid adenoma. We wished to study parathyroid neoplasia further, and in this report we present two independent studies designed to identify new genes involved in the pathogenesis of parathyroid adenomas. First, the genetic and clonal defect resulting from a novel rearrangement in the parathyroid hormone (PTH) gene was characterized, with the possibility of discovering a new oncogene that is placed under the tissue-specific control of PTH. This rearrangement has proven to be a distinct lesion that again implicates PRAD1/cyclin D1 in parathyroid tumorigenesis. Second, we formed the hypothesis that the vitamin D receptor gene could function as a tumor suppressor gene in parathyroid tumors, based on: 1) anti-proliferative effects of vitamin D on parathyroid cells, 2) knowledge of vitamin D regulation of the PTH gene in parathyroid glands, and 3) multiple reports on the effect of the vitamin D system on cancer cell growth. From the study of 32 human parathyroid adenomas, gross rearrangements in the vitamin D receptor (VDR) gene and loss of heterozygosity at this locus do not appear to contribute to parathyroid adenoma neoplasia. From single-strand conformational polymorphism (SSCP) analysis for point mutations in the coding region of VDR cDNA from 31 parathyroid adenomas, our results suggest that point mutations in the VDR structural gene are not likely to participate in tumor pathogenesis.

**Albumin Synthesis after Intense Intermittent Exercise in Human Subjects.** Roger C. Yang, Gary W. Mack, Robert R. Wolfe, and Ethan R. Nadel. Departments of Cellular and Molecular Physiology and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

Endurance training is associated with an expansion of blood volume through a selective increase in plasma volume. This expansion is thought to occur as the consequence of elevated plasma albumin content. Albumin synthesis was measured during the recovery from an intense intermittent exercise protocol shown to expand plasma volume. Using a primed-constant infusion of a stable isotopic tracer of phenylalanine, hepatic fractional synthetic rate (FSR) and absolute synthetic rate (ASR) of albumin were determined from the enrichment of phenylalanine in albumin and in the free intravascular precursor pool. Albumin FSR and ASR were found to be 6.39 ± 0.48 percent/day and 120 ± 9 mg/kg BW/day, respectively, at three to six hours recovery from exercise; the values for FSR and ASR on the time control study day were 5.94 ± 0.47 percent/day and 104 ± 9 mg/kg BW/day, respectively. These significant six percent and 16 percent increases (p < .05) in FSR and ASR, respectively, on the exercise experimental day were associated
with elevated albumin content significant at five and six hours recovery (p < .05), increased total protein content significant throughout recovery (p < .05), and significantly negative free water clearance (p < .05) at two, three and 6.5 hours recovery when compared to baseline values; albumin and total protein content along with free water clearance were not consistently different from their baselines on the time control study day. These findings would all result in the retention of fluid within the circulation after intense exercise. The increase in albumin synthesis measured experimentally could not account for the increase in albumin content seen during recovery from exercise. This finding indicated that an increase in albumin synthesis was not responsible for the acute rise in intravascular albumin content. In contrast, by using a model for movement of albumin between the intra- and extra-vascular compartments and the following assumptions: 1) albumin synthesis during recovery from exercise progressively increases at a linear rate; 2) albumin capillary leak and catabolism are both mass-dependent; and 3) during later stages of recovery, lymph flow does not contribute to the elevation in albumin content, the increase in albumin synthesis measured during the acute recovery from exercise could singly account for expected elevated albumin levels at 24 hr recovery. This model also showed that a decrease in albumin degradation alone could not, produce the albumin content increase at 24 hr recovery and that lymphatic albumin is not a necessary source of albumin for elevation of albumin in the later stages of exercise recovery. Therefore, although the increase in albumin synthesis measured experimentally does not appear to be responsible for the elevation of albumin during acute recovery from exercise, it could account for the increased albumin content seen during the later stages of recovery.

Arthroscopic Reduction and Percutaneous External Fixation of Severe Intra-articular Distal Radius Fractures. Ho H. Yoo and Scott W. Wolfe. Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

Fractures of the distal radius are among the most common injuries treated by orthopaedists today. Early studies implied that distal radius fractures as a group required no special treatment and in general, will result in a functional and pain-free wrist. More recent reports have indicated that severely comminuted intra-articular fractures of the distal radius must be managed aggressively for good long-term outcome. Many authors have argued that management of severely comminuted intra-articular distal radius fracture that is irreducible by external manipulation is open reduction with external or internal fixation. We undertook a study to investigate the role of using percutaneous, arthroscope assisted reduction instead of open reduction in treating these fractures. There may be certain advantages in treating these fractures arthroscopically such as less post-surgical trauma and improved cosmetic, anatomic and functional results. The outcome of seven patients with severe comminuted intraarticular fractures of the distal radius treated by arthroscopic reduction and percutaneous external fixation were retrospectively reviewed. All of the fractures were classified as C3 types under the AO classification scheme. Outcomes were evaluated using the Gartland and Werley functional criteria, an objective wrist exam, a radiographic analysis, and a self-assessment outcome form at an average follow up of 26 months (range 6 to 45 months). All patients were free of pain and had returned to their prior occupation. No patient had articular incongruency of greater than 0.5 mm, and there was no evidence of radiocarpal degenerative change. Active range of motion and grip strength averaged 92 percent and 98 percent, respectively, of the uninjured wrist. The technique of arthroscope-assisted reduction and percutaneous external fixation yielded excellent results in a small group of patients with minimal complications.
Investigation of the Deleted in Colorectal Cancer (DCC) Candidate Tumor Suppressor Gene: Functional Studies and Analysis of a Mutation. Abrahim I. Zafar and Eric R. Fearon. Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

Two independent approaches were undertaken to obtain insights to the role of the Deleted in Colorectal Cancer (DCC) candidate tumor suppressor gene in tumor development. Our first approach was to determine in vitro effects following transfection and expression of the DCC gene in several cell culture lineages. Expression of DCC protein in Rat-1 fibroblasts, following transfection with recombinant DCC mammalian expression constructs, did not alter cell morphology or growth rate; nor did expression of antisense DCC transcript affect cell transformation. We next attempted to express DCC cDNAs in human colon cancer (HCC) cell lines including LoVo, RKO, CaCo-2, HT-29, and SW 116. No cell lines with detectable levels of DCC expression were obtained. This inability to achieve stable constitutive expression of DCC protein may reflect selection pressures against expression of the DCC candidate tumor suppressor in HCC cell lines.

In our second experimental approach, studies were performed to characterize and sequence a deletional breakpoint at the DCC locus in a primary human colorectal tumor (S115). A genomic clone spanning the chromosomal breakpoint region was isolated from a tumor DNA library. Definitive sequence data indicated a tumor genomic rearrangement placing the DCC locus beside an undefined locus containing a Human Line-1 repeat sequence. Further studies of chromosome 18q sequences deleted in tumor S115 may establish the presence or absence of other candidate tumor suppressor genes which are targets of inactivation in human colorectal tumors.

The Effect of Estrogen on the Periventricular Nucleus of the Rat. Molly Zirkle. Yale University School of Medicine, New Haven, Connecticut.

Estrogen has the ability to modify synapses and control neuroendocrine events. GnRH neurons have no estrogen receptors, but the release of GnRH is temporally related to a physiologic estrogen surge. It is hypothesized that estrogen causes the release of GnRH by influencing neural networks connected to GnRH neurons. In the arcuate nucleus and anteroventricular periventricular nucleus of the hypothalamus the physiologic increase in estrogen of the ovarian cycle initiates a cycle of deinnervation/reinnervation of synapses on neuron perikaryal membranes.

In this study, the periventricular region was investigated to see if estrogen influences its synapses. This region is of interest because it contains sexually dimorphic dopamine projections, is estrogen receptor-positive, and contains glial cells which undergo similar estrogen-dependent ultrastructural modifications as those occurring in the arcuate nucleus.

The aim of the study was to determine if there is an estrogen-dependent change in synaptic density of the periventricular region of adult female cycling rats. The periventricular region of animals killed at the peak and nadir of estrogen levels of the estrus cycle was examined using electron microscopy to determine if there was a significant difference in the quantity of axosomatic synapses. We counted synapses on tyrosine hydroxylase (TH)–immunoreactive cells as well as unlabeled cells, and found a reduction of axosomatic synapses on both types of cells during proestrus. The TH cells had a 62.4 percent reduction in axosomatic synapses per 100 micrometers of cell membrane, and the unlabeled cells demonstrated a reduction of 17 percent. Accompanying the reduction of synapses was an increase in the apposition of glial processes to neuronal membranes.