PSYCHOLOGICAL FACTORS ASSOCIATED WITH CONDOM USE AMONG URBAN ADOLESCENTS. Makunda Abdul, Robert W. Ryder, and David L. Katz. Department of Epidemiology and Public Health, Yale University, School of Medicine, New Haven, Connecticut.

The primary risk factor for HIV transmission in adolescents is their sexual behavior, therefore, if we are to decrease the transmission of HIV among adolescents we must address the factors associated with preventive and risky sexual behavior. The goal of this study was to collect information concerning the prevalence and correlates of condom usage in three cohorts of New Haven adolescents.

Research was conducted at three public educational sites. Site 1 was a school for pregnant adolescents. Site 2 was a detention center with educational services for school-aged children. Site 3 was a traditional public high school serving grades 9-12. Students at each site completed a questionnaire assessing sexual behavior, coping skills, self-esteem, substance use, sexual activity and a previous history of physical/sexual abuse. The data collection instrument incorporated three common theories in the field of adolescent sexual behavior: the Health Belief Model, the Self-Efficacy Theory and the Reference Group-Based Social Influence Theory. SAS was used for bivariate analysis.

Site 1 had the lowest rate of condom use, with 29% reporting that they never use a condom. Site 2 reported the highest rate of condom use, with 47% of males and 63% of females reporting that they always used condoms during sexual intercourse. At this site consistent condom use was correlated with a positive attitude toward themselves (p = .001), a belief that they could avoid AIDS if they took precautionary steps (p = .003), and carrying a condom with them "just in case" (p = .001). At site 3, the majority of students used condoms consistently or abstained from sex. Those who did not use condoms consistently were more likely to drink alcohol (p = .042) and report higher levels of stress in their lives (p = .014).

Each educational site had unique characteristics that influenced condom use behavior. These differences should be used to lead future research and the development of better interventions. If we are to stem the tide of adolescent transmission, AIDS education must be targeted, appropriate, and culturally sensitive.

NATURAL PROGRESSION OF AORTIC STENOSIS IN A VETERAN POPULATION. Marjory Alabre and Michael Ezekowitz. Section of Cardiology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

To try to determine the rate of progression of aortic stenosis (AS) and the factors that can help predict its rate of progression, we looked at 109 patients with varying degrees of the disease, selected from the echo lab at the West Haven VA Medical Center. They were reviewed retrospectively and prospectively with respect to a history of hypertension, smoking, cholesterol >200, HDL <29, triglyceride >160, NIDDM, IDDM, BUN >40 and creatinine >2. We looked at two groups, and only those patients with multiple measure-
ments were analyzed. Group I (32 patients) evaluated patients on the basis of aortic valve area (AVA) and group II (69 patients) on the basis of peak aortoventricular (AV) gradient.

Of the 32 patients in group I, 21 (66%) had disease progression over an average of 15 months ± 6 (1 SD). Fifty eight (84%) of the 69 patients in group II showed progression over an average of 4.6 years ± 2 (1 SD). The rates of progression were 0.35 cm²/year + 0.22 (1 SD) for group I and 6 mmHg/year ± 5.5 (1 SD) for group II. The presence of a history of hypertension, smoking, cholesterol >200, triglyceride >160, NIDDM and any two concurrent risk factors were associated with a greater mean AVA decrease, whereas only a history of hypertension and NIDDM were associated with a greater mean peak AV gradient increase. Those results, however, were not statistically significant (p = NS).

We conclude that the rate of progression of aortic stenosis in this population was 0.35 cm²/year ± 0.22 (1 SD) as measured by the AVA and 6 mmHg/year ± 5.5 (1 SD) as measured by the peak AV gradient. In addition, risk factors predicting progression could not be reliably determined because of small sample size.

NEUROCHEMICAL ORGANIZATION OF THE SUBICULAR COMPLEX: NPY/SOM INTERNEURON LOSS IN THE SUBICULUM: A NEW THEORY OF HUMAN MTLE. Kofi Bright Asamadu (Sponsored by Nihal de Lanerolle). Department of Neurosurgery and Neuroscience, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study is to review the neurochemical organization of the subicular complex and to evaluate changes in its organization in human temporal lobe epilepsy patients (TLE), in order to elucidate mechanisms that may contribute to temporal lobe epilepsy. In particular, the cellular morphology and distribution of nonpyramidal interneurons in the human subicular complex are studied to determine if there are any selective losses of these interneurons that could account for epileptogenesis in the human hippocampus.

Brain specimens resected from the hippocampus of patients with refractory TLE were stained for various neurotransmitters and neuropeptides in the subiculum; namely, calbindin, parvalbumin, Gamma aminobutyric acid (GABA), Neuropeptide Y (NPY and somatostatin (SOM). The patients were grouped according to hippocampal pathology; namely, mesial TLE (MTLE), patients with neuronal loss, gliosis and neurochemical reorganization of their hippocampi; Mass-associated TLE (MaTLE patients with mass lesions in their hippocampi; and Paradoxical TLE (PTLE) patients with no evidence of a mass lesion, neuronal loss or reorganization of their hippocampi. Previous analysis of surgical pathology show that the MaTLE and PTLE hippocampi are similar to normal autopsy in the neurochemical organization of their hippocampi, while the MTLE patients showed neurochemical reorganization. Therefore, in this study, the MaTLE and PTLE patients were grouped together as control groups for comparison with the MTLE pathological findings to determine if there were changes in the subicular interneuron groups in MTLE patients that could account for mechanisms underlying epileptogenesis in MTLE.

The subicular complex in the hippocampal slices immunostained for the named interneuron groups were studied and compared among the different epilepsy groups for interneuron morphology and distribution. Neurolucida computer digitizer plots and photomicrographs were used to illustrate and compare the observed patterns among the different epileptic patient groups. The pathology reports as well as some clinical data of all the patients were obtained and reviewed, looking for any patterns among the different epilepsy patients.
The study showed that calbindin, parvalbumin and GABA immunoreactive neurons are preserved in human MTLE, MaTLE and PTLE patients; these different epileptic groups had similar patterns in the distribution and morphology of these interneurons. There was, however, a loss of NPY and somatostatin (NPY/SOM) interneurons in the subiculal complex of MTLE patients compared to the MaTLE/PTLE patients. The pattern of cell loss may involve all sublayers and subregions of the subiculal complex, or it may be patchy and restricted to the layer II cell islands, the pyramidal layer or the polymorphic layer. All the different classes of NPY/SOM interneuron cell types were vulnerable, and the extent of cell loss appeared to correlate with the duration of epileptic activity. This cell loss was accompanied by an intense sprouting of NPY/SOM immunoreactive fibers into all the sublayers and subregions of the subiculal complex, but particularly the molecular layer, and to a lesser extent the polymorphic layer. Compared with MTLE patients, the MaTLE patients demonstrated minimal or no sprouting.

NPY and somatostatin interneurons are inhibitory interneurons that co-contain GABA. Anatomic and physiologic findings of others suggest that these interneurons mediate feedback and feedforward inhibition in the subiculum. In addition, both in vivo and in vitro physiologic studies show that NPY and somatostatin have inhibitory effects as well as antiepileptic activity in the subiculum. Loss of subiculal NPY/SOM interneurons, therefore, could imply loss of important feedback and feedforward inhibitory mechanisms in the subiculum. The accompanying sprouted NPY/SOM fibers could also contribute to epileptogenesis through hyperinnervation of subiculal pyramidal neurons with synchronization of their membrane potentials.

Taking all the data together, we propose an alternative theory for human MTLE; namely, that in MTS, the subiculum is epileptogenic. The spread of epileptic discharges generated in the subiculum through its widespread connections results in the behavioral manifestation of seizures.

**FATIGUE INCREASES SEVERITY OF ACUTE MUSCLE CONTUSION IN A RAT MODEL.** Todd Atkinson, Jacek Cholewicki, Manohar Panjabi and Peter Jokl. Section of Sports Medicine, Department of Orthopedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this project was to determine if a fatigued muscle is more severely injured than a rested muscle when struck by a non-penetrating, blunt object. Using a drop mass technique (ht.=102 cm, wt.=171 grams), reproducible muscle contusions were produced in the gastrocnemius muscles of 14 anesthetized male Wistar rats (10 to 12 weeks of age; 320-360 grams). Each rat had one fatigued and one rested hind limb impacted, allowing each animal to serve as its own internal control. During impact, the force transmitted to the leg and the displacement of the muscle were measured. After isolating the gastrocnemius complex and sciatic nerve, the nerve was stimulated and the muscle’s force generating capacity measured via a load cell. At twitch, tetany, and a fatigue stimulus train, the control leg had significantly greater contractile function suggesting a less severe injury. The force transmitted and the displacement at impact showed no significant difference between the two groups. The data suggests that a fatigued muscle is more prone to a severe muscle contusion but the reasoning cannot be explained by a change in the stiffness of the muscle and its underlying bone.
BRIDGING THE GAP: EFFICACY AND RELEVANCE IN BANGLADESHI MEDICAL EDUCATION. Anna Y. Bloxham, Frank J. Bia and Nora Groce. International Health Program, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The maldistribution of physicians persists as a controversial problem in international health. Since the Alma Ata proclamation described the importance of community-oriented primary care in attaining "Health for All," reform of medical education toward producing providers who will best serve the needs of developing nations has been much discussed but variably implemented. Our study sought to document and discuss some of the complex discrepancies between the healthcare needs of a Dhaka shantytown community ("bostee") and the training, attitudes and professional goals of Bangladeshi medical students studying nearby, contrasting results with the objectives of the Bangladesh National Curriculum and suggesting possible avenues for change.

Interviews were conducted in the homes of 35 women householders in a Dhaka bostee, and complementary questionnaires were received from 59 senior medical students at local government medical colleges. In addition, the author spent several months observing the practices of and discussing these issues with bostee-dwellers, private and public medical students, and physicians and other health care providers active in the teaching and practice of community medicine.

The inhabitants of the study "bostee" have inadequate access to coordinated healthcare, due in part to deficiencies in provider communication, corruption and the lack of a functional urban clinic system. While many of the medical students profess a desire to "help the poor," a socioeconomic gap, a primarily didactic education that underemphasizes and provides little practical exposure to community health and a bias toward specialization and postgraduate training abroad conspire to limit the effectiveness of the official national community medicine curriculum in producing physicians trained and willing to serve underserved communities. Further reorientation and integration of the curriculum, as well as of the health system, is necessary for graduate physicians to more appropriately meet the real needs of Bangladesh as elsewhere in the developing and the developed world.

SOMATOSTATIN SUBTYPE-2 RECEPTOR REGULATION OF GASTRIC ENTEROCHROMAFFIN-LIKE CELL NEOPLASIA IN MASTOMYS NATALENSIS. James F. Borin and Irvin M. Modlin. Gastric Pathobiology Research Group, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The ability of somatostatin (SRIF) and its analogues to inhibit cellular proliferation in animal tumor models has so far translated into only limited success in the treatment of human neoplasms. The mechanism of tumor growth inhibition remains to be fully elucidated. Five somatostatin receptor subtypes (sst1-5) have been cloned recently. By inducing gastric carcinoid tumors of the enterochromaffin-like (ECL) cell in the rodent, mastomys, we sought to determine the sst subtype present, ascertain any receptor mutations or alterations in expression level during neoplastic transformation and characterize the effects of SRIF on ECL cell proliferation in vitro. SRIF receptor cloning from mRNA derived from normal and tumor ECL cells was achieved using gene specific primers. The effect of SRIF on gastrin 10 nM)-stimulated DNA synthesis in vitro was assessed in naive ECL cells. mRNA was quantified using a housekeeping gene as a control, and a Western immunoblot was performed utilizing an sst2-specific antibody. The sst2 cloned from normal and tumor ECL cells encoded a protein of identical amino acid sequence, despite a single bp muta-
tion and demonstrated 88 percent homology with human sst₂. SRIF dose-dependently inhibited DNA synthesis in gastrin stimulated cells (IC₅₀ = 10⁻¹⁰). During neoplastic transformation, there was a decrease in sst₂ expression as measured by a Western immunoblot of gastric mucosa and quantification of mRNA in normal and tumor cells. The sst₂ is present on mastomys ECL cells where it may serve to inhibit gastrin stimulated cellular proliferation. An apparent down-regulation in SRIF receptors during tumorigenesis could contribute to the cell's escape from regulatory control.

**HUMAN CHORIONIC GONADOTROPIN AND RELATED MOLECULES IN PRETERM LABOR.** Tara Lynn Bruce, Yale University School of Medicine, New Haven, Connecticut.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two subunits, α and β, joined non-covalently. Metabolites of hCG include the free α- and β-subunits, nicked hCG and hyperglycosylated hCG. All these forms are detected in serum samples. These same forms plus β-core fragment are also found in the urine.

Preterm labor is a major cause of morbidity and mortality in the perinatal period. hCG has been shown to be an effective marker for Down syndrome pregnancy, ectopic pregnancy and possibly preeclampsia. To test these hypotheses, we measured levels of hCG, hyperglycosylated hCG and β-core fragment in the urine from 72 normal pregnancies and from 18 preterm labor patients. Values were normalized to urine creatinine, and plotted against gestational age, multiples of the normal pregnancy median (MoM) were determined. Our results showed that the preterm labor patient had a lower hCG concentration (0.70 MoM) and a higher proportion of hyperglycosylated hCG molecules (1.5 MoM) than similar gestational age normal pregnancies (not in labor). A similar lowering of hCG levels and raising of the proportion of hyperglycosylated hCG molecules occurs close to term in normal term pregnancy.

These results show that the levels of hCG and hyperglycosylated hCG in preterm labor mimic those seen normally at term. A change in hCG metabolism precedes normal term and preterm labor. Such a change may be involved in the biology of labor, and may be implicated in the initiation of preterm labor.

**THE CORRELATION BETWEEN THE GLUTAMATE RECEPTOR GluR2 AND CALBINDIN-D28k IN THE DEVELOPING GERBIL COCHLEAR NUCLEUS.** Sydney C. Butts and Ilisa R. Schwartz. Section of Otolaryngology, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study was to identify the existence of a relationship between glutamate receptors that regulate calcium influx and calcium binding proteins that buffer intracellular calcium in the developing central auditory system. GluR2 is a subunit of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-preferring glutamate receptors and inhibits calcium influx. Calbindin-D28k (CB) is a prevalent calcium binding protein found in the central auditory system of gerbils. Gerbils at four postnatal ages (7, 14, 21 and 28 days of age) were sacrificed and their brains fixed in preparation for immunocytochemical staining. The cochlear nucleus of gerbils at all four ages were stained with antibodies recognizing GluR2 and CB. The results showed diffuse staining of the cochlear nucleus with the CB antibody at postnatal day 7 (P7). At postnatal day 14 (P14), cells were clearly CB-stained as were their dendritic processes. Many cell types throughout the cochlear nucleus including fusiform cells, cartwheel/stellate cells, octopus
cells, bushy cells and multipolar cells were labeled from P14 through postnatal day 28. GluR2 staining at P7 was prominent in fusiform cells and increased in other cell populations during the second postnatal week. These results suggest that some cells in the cochlear nucleus have low levels of the GluR2 subunit at P7 and might express glutamate receptors that flux calcium. If these cells do flux calcium to a significant degree, as would be expected based on their weak GluR2 expression, the intracellular calcium is not being buffered by CB at P7, whose expression is weak in the cells where GluR2 is also weak. The negative correlation that was hypothesized between the patterns of labeling for GluR2 and CB was not shown. However, there was a significant increase in staining with both antibodies beginning in the second postnatal week which is significant because this is the time when gerbils begin to hear.

INVESTIGATION OF THE DWARFED PHENOTYPE IN TRANSGENIC MICE OVEREXPRESSING THE HUMAN GENE FOR PARATHYROID HORMONE-RELATED PROTEIN. Christi M. Cavaliere, Rupangi C. Vasavada and Andrew F. Stewart. Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Two transgenic mouse lines overexpressing a human parathyroid hormone-related protein (PTHrP) transgene coupled to a rat insulin promoter (RIP) display a dwarfed phenotype. Initial study of this phenotype included measurement of serum insulin-like growth factor-1 (IGF-1), which was reduced in the transgenic mice. Further investigation of the growth axis included Northern blotting and hybridization of liver RNA with an IGF-1 cDNA probe, which showed a decrease in hepatic IGF-1 expression in the transgenic mice. A growth hormone (GH) cDNA probe was used in Northern blotting of pituitary RNA and provided qualitative evidence that GH expression is reduced in transgenic mice compared to normals. Additional Northern blots of pituitary RNA using proopiomelanocortin (POMC) and prolactin (PRL) cDNA probes demonstrated similar expression of PRL and POMC in normal and transgenic mice indicating that panhypopituitarism is an unlikely cause of the dwarfing.

Based on qualitative analyses, isolated growth hormone deficiency is responsible for the dwarfed phenotype of the RIP-PTHrP transgenic mice. While ectopic expression of the PTHrP transgene might disrupt GH production or secretion, PTHrP was undetectable in Northern assays of both the transgenic and normal pituitary. Further studies are necessary to examine the hypothalamus as a possible site of ectopic RIP-PTHrP transgene expression and to investigate the role of PTHrP in the regulation of normal linear growth.

GLUCOCORTICOID SUPPRESSES CBFα1, AN OSTEOBLAST-RESTRICTED TRANSCRIPTION FACTOR REGULATING TYPE I TGF-β RECEPTOR. David J. Chang, Chang-Hua Ji, Sandra Casinghino, Thomas L. McCarthy, Michael Centrella. Section of Plastic and Reconstructive Surgery, Department of Surgery, Yale University, School of Medicine, New Haven, Connecticut.

Transforming growth factor-βs (TGF-βs) are ubiquitous growth regulators that augment bone matrix synthesis. In direct contrast to TGF-β, glucocorticoid suppresses bone formation in vivo and interferes with osteoblast function. In vitro, glucocorticoid reduces TGF-β binding to the type I receptor (TβRI), which is indispensable in mediating its actions and inhibits its stimulatory effects. Osteoblast-enriched cultures prepared from fetal rat calvariae were examined in response to glucocorticoid for changes in TGF-β receptors by
125I ligand binding and Western blot analyses, and for changes in mRNA levels by ribonuclease protection assay. Regulation of TRI gene expression was determined by transfection studies with promoter-reporter constructs. The specific nuclear transcription factors that controlled TRI gene promoter activity were examined by Northern blot, Western blot and electrophoretic mobility shift analysis. The biological consequences of these alterations in receptor levels were assessed by examining the effects of TGF-β treatment on protein synthesis, and on the activity of a TGF-β-dependent promoter-reporter construct.

Glucocorticoid suppressed TGF-β TRI steady-state transcript and protein levels by ~50-90% in a dose- and time-dependent manner. Suppression of TRI expression appeared to occur within a 1.0 kb fragment of the TRI promoter as reporter gene expression directed by the TRI promoter was reduced up to ~50% by 24-48 hr glucocorticoid treatment, while TRI promoter activity was unchanged. The TRI promoter contains cis-acting elements for CBFa1, whose levels increase in parallel with osteoblast differentiation. Glucocorticoid rapidly reduced full-length CBFa1 protein, and decreased nuclear factor binding to oligonucleotide probes with CBFa binding sequences. Glucocorticoid did not, however, influence CBFa1 mRNA levels, suggesting that posttranscriptional events mediated this effect.

Our work provides evidence that glucocorticoid-mediated bone loss may occur in part due to suppression of CBFa1, a tissue-restricted regulator of osteoblast gene expression. A decrease in CBFa1 reduces TRI expression on matrix-producing bone cells, which, in turn, diminishes their functional response to TGF-β.

DEVELOPMENT AND PLASTICITY OF NEUROMUSCULAR CONNECTIONS IN DROSOPHILA. Te Ning Chang. Yale University School of Medicine, New Haven, Connecticut.

This thesis investigates the development and plasticity of neural connections in a simple, well characterized system, the neuromuscular system of Drosophila. The work provides insight into aspects of neuronal pathfinding, target selection, synapse formation and connectivity refinement.

In the main body of the thesis, I investigate perturbations to connectivity in Drosophila embryos and larvae, by using a microbeam laser to ablate motoneuronal cell bodies in the CNS, or to sever peripheral nerves. The embryonic cellular ablations show that despite the stereotypy in normal Drosophila neuromuscular connectivity, denervation induces collateral innervation. These collateral motor endings: 1) arise from neighboring motor endings and nerves; and 2) are physiologically functional. In addition, denervation results in muscle atrophy. The peripheral nerve cuts at different developmental stages show additionally that: 3) the critical period for denervation induced collateral innervation is over by mid-first larval instar; and 4) collateral innervation arises from more distant sources when local sources are not available. Furthermore, post-nerve cut timing and distribution of expression of synapse associated proteins including synaptotagmin, Fasciclin II, β-integrin and a putative glutamate receptor were characterized.

An antibody to a vertebrate glutamate receptor subunit, GluR1, was discovered to give robust and specific labeling at the neuromuscular synaptic site, where glutamate is the major neurotransmitter. Analysis of a mutant for synaptotagmin, which reduces postsynaptic response, showed a reduction in GluR1 IR as well as IR for another vesicle associated protein, synaptobrevin, although initial localization of GluR1 IR at the motor ending in the synaptotagmin mutant embryo was not affected.

In the Appendix, I describe studies that address normal development of Drosophila neuromuscular connections. Techniques used include live visualization of in situ growth
cone dynamics in *Drosophila* embryos, dil backfills from embryonic muscle fibers, and immunolocalization of a NMDA receptor-like protein. The results show that: 1) there is variability in innervation frequencies and target choices that must be considered when evaluating deviations from the norm; 2) CNS arborizations can also show high stereotypy and indicate close associations with homologous motoneurons in the same and adjacent segments; and 3) motoneuronal filopodia are extremely dynamic, and may transiently contact a large fraction of the muscle fibers in their own and adjacent segments.

**NITRIC OXIDE PRODUCTION BY ENDOTHELIAL CELLS (EC) IN AMBIENT STATIC OR PULSATILE PRESSURE IN VITRO.** Henry Chen, Mark Awolesi, Angela Vouyouka, Carol Sawmiller, Joseph Ricotta and Bauer E. Sumpio. Section of Vascular Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

Hemodynamic forces have been shown to influence endothelial nitric oxide synthase (eNOS) expression and activity. There is also evidence that nitric oxide can affect cell proliferation. Meanwhile, we have previously observed that high ambient static (135 mm Hg) and pulsatile (160/110 mm Hg) pressure decrease endothelial cell proliferation compared to EC exposed to atmospheric pressure (control). We sought to test the hypothesis that this decrease in EC proliferation at high ambient pressure conditions is due to enhanced nitric oxide (NO) production. Cultured bovine aortic EC were seeded onto 12-well plates (2000 cells/well) and allowed to attach for 24 hours. Cells were maintained in Dulbecco's Modified Eagle Media supplemented with 10 percent calf serum and substrates. EC were placed in custom designed pressure chambers maintained with 5 percent CO$_2$/air at 37°C and exposed to either atmospheric, static (135 mm Hg) or pulsatile pressure (160/110 mm. Hg) at a frequency of 60 cycles/min. On days 1, 3, 5 and 7 the cells were fixed with 4 percent freshly polymerized paraformaldehyde/0.1 M phosphate buffer solution. Nitric oxide synthase within the EC was then visualized by NADPH diaphorase staining. The results indicate that eNOS expression is increased in EC exposed to static or pulsatile pressure compared to EC maintained in atmospheric pressure. These findings are consistent with preliminary evidence that EC placed under static (105 min Hg) and pulsatile pressure (120/90 mm Hg) exhibit greater transcription of the nitric oxide synthase gene. Next, proliferation of EC placed in atmospheric, static (135 mm Hg) or pulsatile pressure (160/110 mm Hg) was assessed in the presence or absence of the nitric oxide inhibitor, No-nitro-L-arginine methyl ester (L-NAME) (100 mmol/L) or its inactive enantiomer, D-NAME (100 mmol/L). Media was changed and appropriate agent was replenished on days 2, 4 and 6. On days 1, 3, 5 and 7, EC proliferation was determined with a Coulter counter after trypsin release. There was no demonstrable difference in the EC growth curves in the presence or absence of L-NAME or D-NAME under the different pressure conditions. Regardless of agent added, EC grown under pulsatile and static pressure had a slower proliferation rate than EC maintained in atmospheric conditions. We hypothesize that while eNOS is expressed more at high static and pulsatile pressure, the decrease in EC proliferation seen under these pressures is not due to increased NO activity.
CORRELATION BETWEEN CLINICAL PRESENTATION AND ULTRASTRUCTURAL PATHOLOGY IN PRIMARY CILIARY DYSKINESIA. Gregory Y. Chin, Michael Kashgarian*, David E. Karas. Section of Otolaryngology, Department of Surgery and *Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

Primary ciliary dyskinesia (PCD) represents a spectrum of ciliary defects that lead to impaired ciliary motion and result in respiratory tract dysfunction and recurrent infection. Few studies of PCD have correlated clinical presentation with ultrastructural findings. Such data could reduce the number of biopsies and electron microscopies that are performed by enabling the clinician to rule out PCD by presentation alone. This would lower patient morbidity and cost. We reviewed the records of 118 patients with ciliary biopsies or brushings examined at Yale from 1991 to 1998. Patients who presented with cough alone were highly unlikely to have PCD (chi-square 24.85, p <.0001). In contrast, patients who presented with multiple manifestations were highly likely to have PCD (chi-square 22.2, p <.0001). Furthermore, we provide 13 additional cases of PCD with random ciliary orientation as the sole cause that presented similarly to those of other ultrastructural defects. This information may assist clinicians in deciding if patients merit ciliary biopsy with electron microscopy or other evaluation.

MATERNAL SERUM URIC ACID LEVELS IN TWIN AND TRIPLET GESTATIONS COMPLICATED BY PREECLAMPSIA. Kent H. Chou, Yuk-Kwang Chung, In-Sik Lee, Joshua A. Copel and Chaur-Dong Hsu. Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study was 1) to compare mean maternal serum uric acid levels in preeclamptic and non-preeclamptic twin and triplet gestations, 2) to establish uric acid cutoff values for the identification of preeclamptic twin and triplet gestations and 3) to compare mean uric acid levels at different stages of gestation in preeclamptic and non-preeclamptic twin gestations.

To this end, we identified 106 twin and 23 triplet gestations in which uric acid measurements had been recorded. Fifty-five of the twin gestations and 15 of the triplet gestations were complicated by preeclampsia. We then calculated mean uric acid levels in preeclamptic and non-preeclamptic twin and triplet gestations, generated receiver operating characteristic curves for twin and triplet gestations, and determined mean uric acid levels at different stages of gestation in preeclamptic and non-preeclamptic twin gestations.

Mean uric acid levels in preeclamptic twin and triplet gestations were significantly higher than in their non-preeclamptic counterparts. Uric acid levels of 6.3 and 6.8 mg/dL were the optimal cutoff values for the detection of preeclampsia in twin and triplet gestations, respectively. Mean uric acid levels at all gestational ages beyond 24 weeks were significantly higher in preeclamptic twin gestations than in non-preeclamptic ones.

We conclude that mean uric acid levels in preeclamptic twin and triplet gestations are significantly higher than in their non-preeclamptic counterparts. Cutoff values of 6.3 and 6.8 mg/dL in twin and triplet gestations, respectively, may be used to assist in the identification of preeclamptic pregnancies. Mean uric acid levels rise during both preeclamptic and nonpreeclamptic twin pregnancies and are greater at all gestational ages beyond 24 weeks in twin pregnancies complicated by preeclampsia.
AN ANALYSIS OF ISSUES SURROUNDING DISCLOSURE OF THE HIV DIAGNOSIS TO CHILDREN. Reshma R. Chugani, Kimberly Freudigman, Anne Murphy, Warren A. Andiman. Department of Pediatrics, Yale University, School of Medicine, New Haven, Connecticut.

Disclosure of a medical diagnosis to children has been shown to be beneficial in terminal and chronic illnesses of childhood. Most HIV-infected children do not know their diagnosis, and disclosure has not been well-studied in this population. We sought to describe the demographic, clinical, and psychosocial characteristics of a cohort of HIV-infected children and to identify those variables associated with disclosure of the diagnosis to these children by their caretakers.

A retrospective review was conducted of 70 children older than five years of age with perinatally acquired HIV infection. Personal interviews with a subset of caretakers of these children (n = 23) were conducted to produce vignettes that would detail their experiences with HIV disease specifically related to the issue of disclosure.

There were 70 children in the cohort. At the time of the study, 74% (52/70) of patients were alive and 26% (18/70) were deceased. Diagnosis was disclosed to 26% (18/70) of patients at a mean age of 107 months (8.9 years). Univariate analysis revealed five variables associated with disclosure: older age of the child ($\chi^2 = 7.68, p = .0085$), a primary caretaker not biologically related to the child ($\chi^2 = 3.25, p = .0485$) and an older age at AIDS diagnosis ($t = 2.60, p = .0232$). These five variables accounted for 74% of the variance between disclosed and undisclosed groups as determined by a multiple log regression analysis. In citing reasons for disclosure or nondisclosure to the child, caretakers focused on the potential consequences for the child rather than for the caretaker or the family.

Thus, disclosure of HIV diagnosis to children is a complex process, and the family’s decision to disclose or not is personal and deliberate. In this population, five identified variables together predicted disclosure or nondisclosure in nearly 75% of cases.

THE ANALYSIS OF THE ADHERENCE/INVASION OF Trypanosoma cruzi TO HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS IN VITRO AND IN SITU. Helen Mi-Hae Chun (Sponsored by Joseph A. Madri). Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

Trypanosoma cruzi, the causal agent of Chagas’ disease, is an obligate-intracellular protozoan parasite. It is estimated that over 16 million people in the Central and South American continent are infected with this protozoan. The mechanisms governing T. cruzi-endothelial cell adhesion and invasion have not been elucidated but certainly represent a critical step in the ensuing disease process. The present study assesses the interaction of T. cruzi with vascular endothelium in vitro and in situ. Previous work (Schenkman et al., 1991) using fibroblasts demonstrated the use of glutaraldehyde fixation of cells to analyze the attachment phase of T. cruzi infection. We apply this system to the more appropriate targets, endothelial cells. The experiments in this project were designed to separate and quantify the events of adhesion and invasion (internalization) of endothelial cells and to characterize these processes at the ultra-structural level using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The release of inflammatory cytokines has been described during the acute phase of Chagas’ disease (Hoft et al., 1993, Truyens et al., 1994, Torricio et al., 1988, Mc Cabe et al., 1991). We propose that cytokines involve the participation of surface molecules that T. cruzi may exploit to adhere to one of their major cellular targets, the endothelium. We tested the hypothesis that
endothelial cell activation followed by exposure to TNFα makes the endothelial cells more susceptible to T. cruzi adherence and invasion. Results from these studies demonstrated: 1) T. cruzi successfully bind to and invade HUVECs. The infection of HUVECs, which are considered non-professional phagocytes, is suggestive of an endocytic/phagocytic mechanism as demonstrated by the presence of trypanosomes within parasitophorous vacuoles. 2) Glutaraldehyde-fixed endothelial cells bind T. cruzi with kinetics similar to untreated cells and may be used for analyzing the process of adherence. 3) TNFα treatment of HUVECs significantly increases T. cruzi adhesion to endothelial cells. 4) Adherence and invasion appear to be independent events as demonstrated by an increased in adhesion without a significant increase in internalization in TNFα-treated HUVECs. A greater understanding of the parasite host-endothelial cell interaction would facilitate investigations of cell surface molecules that are involved in this interaction. This understanding would enable the design of therapeutic molecules to prevent adhesion and thereby possibly prevent T. cruzi infection.

AN INNOVATIVE CURRICULUM OF LITERATURE AND MEDICINE DURING RESIDENCY EDUCATION: POETRY ON THE WARDS. Pieter A. Cohen (Sponsored by Auguste H. Fortin, VI). Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

This study assessed the introduction of an innovative, modular, case-based literature and medicine curriculum onto the wards during internal medicine residency education. From July to October 1997, internal medicine teams, including seven attending physicians and 31 residents and students, participated in twice-weekly case-based poetry discussions at two community teaching hospitals in Waterbury, Connecticut. Attending physicians, residents and students completed anonymous questionnaires both prior to and following participation. Results were analyzed using two-tailed t-tests and chi-square analysis. Seventy-nine percent of the residents and students and 100 percent of the attending physicians found the modular curriculum to be useful. Thirty-nine percent of the residents and students and 43 percent of attending physicians felt that the discussions had addressed specific clinical situations on the wards. After participating, the attending physicians agreed that time devoted to the humanities and medicine on the wards could improve house officers' abilities to talk to, understand and/or empathize with their patients (mean 3.7/5.0, SD 0.8 on a 5-point Likert scale, where "1" = "strongly disagree" to "5" = "strongly agree". Qualitative data revealed that the discussions succeeded in introducing humanistic aspects of the practice of medicine during attending rounds. However, participants' attitudes toward time devoted to the humanities and medicine did not change significantly over the course of the intervention. The barriers identified by both attending physicians and residents to introducing the curriculum included lack of time, negative resident attitudes and lack of perceived relevance to clinical practice. Solutions for overcoming these barriers include: 1) less frequent and more selective use of the curriculum in both the inpatient and outpatient settings; 2) further faculty development focused on teaching of the humanities in residency education; and 3) further research of residents' attitudes towards introducing the humanities and medicine during residency training.
CLONING, SEQUENCING AND PARTIAL CHARACTERIZATION OF THE 5' FLANKING REGION OF THE MOUSE NEUTROPHIL COLLAGENASE GENE: CONSERVATION WITH THE HUMAN NEUTROPHIL COLLAGENASE REGULATORY REGION. Marcoli Cyrille, Nathan Lawson, Theresa Zibello and Nancy Berliner. Department of Internal Medicine, Section of Hematology, Yale University School of Medicine, New Haven, Connecticut.

The expression of the secondary granule protein (SGP) genes lactoferrin (LF), transcobalamin (TBI), human neutrophil gelatinase (HNG) and collagenase (HNC) is coordinately regulated at the level of mRNA transcription. Our laboratory has previously identified a silencer element in the LF promoter that binds CCAAT displacement protein (CDP); when CDP is overexpressed in myeloid cells, it blocks the expression of all SGP genes. CDP is a ubiquitous repressor known to be implicated in the regulation of many myeloid-specific genes, and we wish to determine the means by which it acts as a stage specific repressor of multiple genes at different stages of differentiation. Here we describe the cloning, sequencing and partial characterization of the 5' flanking region of the mouse neutrophil collagenase (MNC) gene. The MNC promoter shows a high degree of conservation over more than 200bp with the human homolog, with a 56bp stretch sharing 81.4 percent nucleotide identity. Within this region, we have identified well described control elements involved in myeloid gene regulation, including a canonical consensus sequence for the CCAAT enhancer binding protein alpha (CEBPα). Functional assays will be necessary to establish the functional significance of these regions. CDP binding sites are highly promiscuous and can only be characterized functionally. The identification of the promoter will now allow such characterization.

POWER AND MYSTICISM IN THE INTRODUCTION OF ANESTHESIA IN NINETEENTH CENTURY AMERICA. Naomi Donnelley (Sponsored by Maria Trumpler). Department of the History of Medicine, Yale University School of Medicine, New Haven, Connecticut.

The introduction of the inhalational anesthetics to surgery in nineteenth century America revolutionized the treatment of pain. It promised relief from the horror of experiencing every movement of the surgeon’s knife. Beyond pain relief, however, what was anesthesia’s legacy to the American medical profession? Anesthesia was a new tool in the medical arsenal that possessed the luster of science, and at the same time, possessed ill-defined mystical qualities—ether, chloroform and nitrous oxide could induce an artificial death from which patients could be “resurrected.” In this thesis, I argue that anesthesia’s marriage of technology and mysticism had an enormous transformative power that helped redirect the medical profession and change the nature of the doctor/patient relationship. In support of my argument, I examined both medical and lay responses to the anesthetics, paying careful attention to the emotional, cultural and philosophical concerns the new anesthetics raised. As wielders of the mystically and symbolically charged new tool, doctors were forced to address not only the scientific, but also the metaphysical implications of the anesthetics. They became philosophers and moral and social caretakers, as well as physical healers. As their authority expanded, so did their confidence and prestige.
URINARY CATECHOLAMINES AND CORTISOL IN ATTENTION DEFICIT/HYPERACTIVITY DISORDER. Marcia Dover, John Holahan, George Anderson, Karen Marchione, Sally Shaywitz and Bennett Shaywitz. Department of Pediatrics, Yale University, School of Medicine, New Haven, Connecticut.

Our aim was to examine urinary catecholamines and cortisol excretion in a large sample of children with attention deficit/hyperactivity disorder (ADHD) of varying subtypes, looking for differences between the subtypes and with respect to normal controls. We also sought to establish the effect of comorbid learning disorder (LD) or oppositional defiant disorder/conduct disorder in ADHD, and to replicate previous findings regarding urinary epinephrine excretion in ADHD. Boys (n = 373) between 7.5 and 13.5 years of age were recruited. Tests of attention, impulsivity and visio-spatial tasks were administered during the course of a three-hour urine collection period. Urinary excretion rates for norepinephrine, epinephrine, dopamine and cortisol were determined. A two-way ANOVA was used to examine urinary epinephrine excretion in children with ADHD compared with normal children. A MANOVA was used to examine possible relationships between the catecholamines and ADHD, and ANOVA was used to examine possible relationships between cortisol and ADHD. Urinary epinephrine levels were significantly lower in children with ADHD compared with normal children (F1,370 = 5.88, p = .016). There was a significant interaction between ODD/CD status and ADHD in urinary catecholamine levels (Wilk’s λ = .950, F9,864 = 2.054, p = .03 1), which was attributable to multiple simple interactions between the AD/HD-HI sub-type and the other ADHD subtypes. There was a significant main effect of ADHD subtype on urinary catecholamine levels (Wilk’s λ = .943, F9,864 = 2.054, p = .013), with dopamine levels significantly increased in the AD/HD-HI group. There were no significant differences in cortisol values among the ADHD subtypes and the control group. Lower urinary epinephrine excretion, without a change in urinary cortisol excretion, may be associated with ADHD. This finding is compatible with theories of abnormal sympathetic/adrenomedulary stress response systems in ADHD.

DEVELOPMENTAL REGULATION OF EXPRESSION OF KSP-CADHERIN IN THE HUMAN KIDNEY. K.E. Earle, R.C. Kim, C.-L. Yang, R.B. Thomson, P.S. Aronson. Department of Medicine, Yale University School of Medicine, New Haven, Connecticut.

Cell adhesion molecules, including the cadherins, are important regulators of cellular events including differentiation and proliferation. Ksp-cadherin is a novel member of the cadherin family expressed only in the kidney (Thomson et al., JBC 270:17594, 1995). Monoclonal antibodies were generated against a maltose-binding-protein fusion protein incorporating the C-terminal. 266 amino acid residues of human Ksp-cadherin. Hybridomas were initially screened by ELISA to the fusion protein compared to the maltose-binding-protein, and then tested for reactivity with human Ksp-cadherin by Western blot analysis and immunofluorescence. Two clones were isolated by limiting dilution, one of which (4H6) was used for subsequent studies. Specificity of this antibody was confirmed by Western blot analysis demonstrating labeling of a 130 kDa protein in L-cells transfected with human Ksp-cadherin but not in control cells. The labeled protein in transfected cells was identical in size to that labeled in native human kidney membranes.

Monoclonal antibody 4H6 was used to characterize the expression of Ksp-cadherin in formalin-fixed, paraffin-embedded human kidney sections that were stained using microwave antigen retrieval techniques. By immunofluorescence microscopy, Ksp-cad-
herin staining was detected on the basolateral membrane of multiple segments of the nephron. In the cortical region, distal tubules were strongly labeled, whereas staining of proximal tubules was variable and of weaker intensity. Intermediate staining was found in cortical-collecting ducts. No staining was detected in the glomeruli. In the medullary regions, the highest level of staining was found in thick ascending limbs of the loop of Henle. Medullary collecting ducts had an intermediate level of staining, whereas thin limbs of the loop of Henle were weakly labeled, if at all.

We also characterized Ksp-cadherin expression during human kidney development. Tissue sections were obtained from kidneys at 14 to 20 weeks of fetal development. In general, there was a gradient of staining with greater labeling of more differentiated compared to less differentiated structures. Staining was greatest for collecting tubules, with intermediate staining of ureteric ducts, and minimal staining of ureteric buds.

THE VALUE OF VESTIBULAR FUNCTION TESTING TO PREDICT DISEQUILIBRIUM AFTER ABLATIVE VESTIBULAR SURGERY. Mark R. Homicz, John F. Kveton. Section of Otolaryngology, Department of Surgery, Yale University, School of Medicine, New Haven, Connecticut.

Disequilibrium after ablative vestibular surgery is a sign of poor vestibular compensation and suggests central vestibular dysfunction. In an attempt to predict disequilibrium after ablative vestibular surgery, a vestibular function battery (electronystagmography, rotational vestibular testing and dynamic posturography) was performed on 24 patients with peripheral vestibular disorders prior to surgery. Following labyrinthectomy or vestibular neurectomy, patient symptoms were assessed by review of patient records from follow-up visits as well as by telephone survey. Sixty percent (3/5) of patients who displayed central and peripheral findings described disequilibrium preoperatively. After surgery, disequilibrium worsened or was unchanged in 80 percent (4/5) of patients. Fifty-three percent (10/19) of patients without central findings described disequilibrium prior to surgery. Postoperative disequilibrium was present in 32 percent (6/19). The observed difference in postoperative disequilibrium between the groups with and without central findings did not reach statistical significance (P = .16) Thus, this study suggests, but was unable to confirm, the utility of the vestibular test battery for prediction of poor vestibular compensation postoperatively.

THE EQUIVALENCE OF SINGLE AND INCREMENTAL TRAUMA IN THE RABBIT ANTERIOR CRUCIATE LIGAMENT. Russel C. Huang and Manohar M. Panjabi. Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

In musculoskeletal trauma modeling, utilization of multiple trauma increments of increasing intensity permits superior determination of injury thresholds, better understanding of injury progression from mild to severe, and minimization of the number of specimens required when compared to single trauma techniques. The purpose of the present study was to determine if incremental and single ligamentous trauma are biomechanically equivalent and to determine which techniques for detection of ligamentous injury are more sensitive. Eleven paired fresh rabbit bone-anterior cruciate ligament-bone preparations were used. One of each pair (control) was stretched to 88 percent of the average failure deformation (Dfail), and then stretched to failure. The other ligament (experimental) was incrementally stretched to 55, 66, 77 and 88 percent of the average Dfail, and
then stretched to failure. Stress relaxation tests quantified ligament viscoelastic properties before each injury cycle. The load-deformation curves collected during injury cycles were characterized by eight parameters: failure force ($F_{fail}$), failure deformation ($D_{fail}$), energy to failure ($E_{fail}$), deformations ($D_5$, $D_{10}$, $D_{25}$ and $D_{50}$) measured at 5, 10, 25 and 50 percent of $F_{fail}$, and stiffness ($K_{50}$) measured at 50 percent of $F_{fail}$. Stress relaxation curves (force vs. time) were fitted to a three-element viscoelastic model. This model consisted of a spring with stiffness coefficient $k$, in series with a parallel spring and dashpot with stiffness and damping coefficients of $k_2$ and $C_2$, respectively. Two-tailed paired Student's $t$-tests at a $p < .05$ level failed to reveal any statistically significant differences in the load-deformation or viscoelastic properties of the control and experimental ligaments. ANOVA of consecutive trauma increments applied to the experimental ligaments showed that load-deformation curves were more sensitive than viscoelastic properties in the detection of incremental injury. In conclusion, our study indicates that incremental and single ligamentous trauma are biomechanically equivalent, and that changes in load-deformation curves are the more sensitive indicator of injury.

T CELL RECEPTOR DNA VACCINATION AGAINST MURINE T CELL LYMPHOMA. Daniel J. Hurwitz, Michael Girardi, Renata Filler, Robert Tigelaar and Richard Edelson. Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut.

Malignant cutaneous T cell lymphoma (CTCL) cells are clonal with a distinct T cell receptor (TCR). Anti-tumor immune responses can theoretically be generated by inoculation with TCR-DNA expression plasmids encoding clone-specific TCR gene segments. The 2B4.11-(B10.A x AKR)$F_1$ system is an established murine model of T cell lymphoma. In this model the subcutaneous injection of $5 \times 10^6$ 2B4.11 T cell hybridoma cells into syngeneic (B10.A x AKR)$F_1$ mice results in lethal tumor growth within weeks.

DNA vaccines may offer the opportunity to generate stronger cellular immune responses against peptides encoded by injected DNA as compared with conventional protein vaccines. T cells, including the 2B4 T cell hybridoma used in these studies, express on their surface a unique T cell receptor (TCR). The TCR from a clonal T cell line, such as 2B4, presents a unique antigen for recognition and attack by other T cells.

Inoculation with TCR-DNA plasmids encoding portions of the a chain of the 2B4 TCR elicited specific anti-2B4.11 immune responses, as determined by in vitro proliferation assays. Specifically, lymphocytes from (BIO.A x AKR)$F_1$ mice immunized with DNA plasmids encoding portions of the 2B4.11 T cell hybridoma. TCR preferentially proliferated when stimulated by 2B4.11 cells versus other syngeneic hybridomas not sharing the 2B4.11 TCR (i.e., the C10.9 cell line).

The ability to target an undesirable T cell clone by immunizing against its TCR could be useful in treating not only T cell tumors such as cutaneous T cell lymphoma (CTCL), but also other T cell mediated conditions, such as graft versus host (GVH) and autoimmune diseases.
RETINOIC ACID MODULATES C-FMS PROTO-ONCOGENE EXPRESSION IN HORMONE-INDEPENDENT HUMAN BREAST CARCINOMA CELLS. Susan S. Kim and Maryann B. Flick (Sponsored by Barry M. Kacinski and Eva Sapi). Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut.

We investigated the possibility of differential regulation of c-fms gene expression by retinoic acid (RA) in hormone-dependent and hormone-independent breast carcinoma cells. Northern blot analysis demonstrated that RA significantly increases fms transcript levels in a time-dependent manner in two advanced (estrogen receptor-negative, hormone-independent, high metastatic potential) breast cancer cell lines, SKBR3 and BT20, but has no significant effect on fms transcript levels in MCF7, a cell line exhibiting an "early" phenotype (estrogen receptor-negative, hormone-dependent, low metastatic potential). The RA-induced increase in fms transcript levels was blocked by RO41-5253, a selective RA receptor α(RARα) antagonist, suggesting that fms induction by RA may be mediated by RARα. DNase I “footprinting” studies revealed RA-mediated time-dependent changes in the appearance of a protected band in an activator protein-1 (AP-1) site. The pattern of protection after RA treatment, associated with changes in the nature of protein binding at the AP-1 site, indicates that RAR-AP-1 interaction may play an important role in the two- to three-fold upregulation of first promoter activity by RA in SKBR3 cells observed in our transient transfection analysis. Our results offer an interesting counterpart to the observation that breast cancer cell lines representing later stages of tumor progression tend to be resistant to the growth-suppressive effects of retinoids. Since increased CSF-1/CSF-IR expression in breast cancer cells is associated with invasiveness and higher metastatic potential, the induction of fms transcription by RA suggests that retinoids may play conflicting roles throughout breast cancer progression, inhibiting proliferation at earlier stages and possibly promoting malignant transformation at later stages of breast cancer.

HYPERGLYCOSEYLATED HUMAN CHORIONIC GONADOTROPIN IN PREECLAMPSIA. Jessica M. Kingston and Laurence A. Cole. Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone made by trophoblasts of the placenta. Hyperglycosylated hCG (H-hCG) is an aberrant form of hCG and represents a small amount of hCG molecules produced in normal pregnancies. Changes in H-hCG levels can modulate hCG production. Raised hCG levels are found in cases of preeclampsia. We investigated the relationship between H-hCG and hCG levels in this disorder. H-hCG and hCG levels were measured in urine samples from 71 controls, 14 preeclampsia cases and three gestational hypertension cases using specific enzyme-linked immunospectrometric assays (ELISA). We also calculated the proportion of H-hCG molecules (%H-hCG) and correlated all results with gestational age. hCG levels were significantly higher, 4.3 multiples of control median (MoM), and %H-hCG lower, 0.28 MoM, in the preeclampsia cases (p <.001 and p = .001, respectively). Using bivariate analysis, the tests together detected 71 percent of preeclampsia cases at a 5.6 percent false-positive rate. We also tested 124 controls and 18 prospective preeclampsia cases from the second trimester. While no statistical difference was found in hCG results, significantly reduced %H-hCG values (0.44 MoM, p <.02) were detected before disease was evident. hCG and %H-hCG measurements may be useful for detecting preeclampsia, and possibly for predicting the disease before it is clinically evident. Larger studies are warranted to confirm these results.
AN EVALUATION OF A METHOD TO SPREAD GENETICALLY ALTERED BACTERIA IN THE PREVENTION OF CHAGAS' DISEASE. Andrew T. Kroger, Frank F. Richards and Ravi Durvasula. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Chagas’ Disease is an infectious disease that afflicts 19 to 24 million people in Latin America. The infectious agent is a parasitic protozoan Trypanosoma cruzi, which is spread to animals and humans by an insect vector known as a Reduviid bug. This thesis proposes an approach to the prevention of Chagas’ disease. One species of the Reduviid Bug vector, Rhodnius prolixus, contains Rhodococcus rhodnii. These bacteria are commensal, acquired from natural surroundings soon after hatching, and have found a permanent niche in the bug. Both this bacterium and the T. cruzi parasite occupy the hindgut of R. prolixus. It has been possible to alter the genome of this bacterium so that it expresses proteins detrimental to the parasite. The medium in which our GAS is spread to the insect is a simulated feces preparation called CRUZIGARD. This method takes advantage of the natural coprophagic or feces-probing behavior of 1st and 2nd nymphal instar stages of R. prolixus.

We find that at least one type of GAS can be transferred from CRUZIGARD to R. prolixus when applied once a month. This GAS is successfully recovered from the insect hindgut after previous exposure, and verified by PCR. A single application of this same type of GAS is less successful in transferring the bacteria.

GENE THERAPY OF CANAVAN DISEASE. Kimara J. Leibowitz, Matthew J. During, Paola Leone and Margretta R. Seashore. Gene Therapy and Neurogenetics Laboratory, Department of Neurosurgery, Yale University School of Medicine, New Haven, Connecticut.

Canavan disease is an autosomal recessive leukodystrophy associated with mental retardation and spongy degeneration of the brain resulting in death. The purpose of this study was to evaluate the efficacy and safety of the gene therapy of Canavan disease. Male Fischer 344 rats were anesthetized and placed in stereotactic frames for either intraparenchymal or intracerebroventricular injections of the vector, liposome-polymerDNA (LPD), complexed with the gene of interest, asparatoacylase (ASPA). The E. coli β-galactosidase reporter gene, otherwise identified as lacZ, served as a histological marker of gene expression. Variable volumes of the LPD complex were administered ranging from 2-50 µl at a DNA concentration of 300 µg/ml. Following sacrifice, gene expression was identified through visualization of the marker gene, LacZ, as indigo blue cells. Indirect evaluation of the ASPA gene expression was detected through RT-PCR. All the subjects that were injected with LPD/lacZ demonstrated gene expression through positive staining of the β-galactosidase enzyme at one month, three months, and six months following the surgery. Approximately 80 percent of the animals also stained positively for the β-galactosidase at nine months postinjection. The RT-PCR experiments demonstrated widespread expression of the ASPA gene for ten months following intracerebroventricular injections. In addition, there were no abnormal physiological and behavioral responses to the LPD injection observed. The data suggest that a clinically sufficient quantity of the protein was produced in the brain parenchyma given the injection volumes and concentration of DNA used. The animal studies described in this work helped to form the foundation for the subsequent primate and eventual human studies. Currently, Phase I clinical trials are being conducted at the Children’s Clinical Research Center (CCRC) at Yale-New Haven Children’s Hospital.
DELIBERATE WATER STORAGE PRACTICES AND PREVALENCE OF Aedes aegypti IN THE AMAZON REGION OF PERU. Lisa S. Lipschitz, Douglas M. Watts and Mark L. Wilson. Yale University School of Medicine, New Haven, Connecticut and United States Naval Medical Research Institute Detachment, Lima, Peru.

This study examined how water use, source and storage affected Aedes aegypti larval contamination in the Amazon region of Peru. Standardized interviews and larval mosquito sampling were performed in a random sample of homes in the urban city of Iquitos (Punchana), in the jungle village of Porvenir and in the rural town of Primavera. Results revealed that homes were more likely to harbor larvae in cleaning water (9.4%) than potable water (1.4%). Bivariate analysis of cleaning water revealed that larval presence was associated with location (p = .012), length of residence (p = .007), primary water source (p < .001), secondary water source (p = .002), water availability (p < .001), water reliability (p = .007), storage container (p < .001), duration of storage (p < .001), quantity of stored water (p < .001), dirty containers (p < .001) and uncovered containers (p = .012). Multivariate analysis demonstrated that cylinders (p < .001), storage time (p < .001) and dirty containers (p < .001) independently contributed to presence of larval A. aegypti in containers of cleaning water. Storage of rain water, which contributed to all three aforementioned variables, was most often used in homes with an unreliable water source (p = .049). The most unreliable source was piped water, followed by wells and then rivers. Cylinders were associated with increased storage time, dirty appearing containers and larval presence. People used cylinders because of their large size (83 percent) and did not use them because of cost (45 percent). In conclusion, cleaning water was most likely to harbor A. aegypti larvae as compared to potable water. Infested cleaning water appeared to result from an unreliable water source leading to rain storage in barrels for longer periods of time without concern for hygiene. If the economic status of this region improves without changes in water reliability, more people will be able to afford cylinders for storing rain water. Ironically, this could result in an increase in A. aegypti, and possible risk of dengue.

A PROSPECTIVE STUDY OF NEONATAL WITHDRAWAL IN INFANTS EXPOSED TO COCAINE AND METHADONE. Barbara A. McGee and Linda C. Mayes. Child Study Center, Yale University School of Medicine, New Haven, Connecticut.

The purpose of the present study was to prospectively examine the following hypothesis: Cocaine use among pregnant women maintained on methadone increases the duration and the severity of the neonatal abstinence syndrome (NAS) among their infants. A group of 23 infants born to mothers participating in a methadone maintenance program were followed for severity of NAS after poly-drug exposure in utero. Severity of withdrawal was indexed by infant length of stay in the hospital, duration of treatment with medication, first and maximum symptom scores and the dosages of withdrawal medications used. During pregnancy, information was gathered on maternal daily methadone dose, urine toxicology, pregnancy complications and medication use. Fourteen (61 percent) of the mothers in the sample had positive urine screens for cocaine, and five (22 percent) took benzodiazepines regularly during pregnancy. Infants exposed to both cocaine and methadone tended toward a shorter duration of therapy, however, the differences were not found to be statistically significant (F = 1.54 , p = .23). There was a positive correlation between maternal methadone dose and the number of days infants spent on the highest doses of withdrawal medication (r = .53, p ≤ .01). In addition, benzodiazepine
exposure significantly contributed to longer infant hospital stays (F = 8.3, p < .01), and duration of medication treatment (F = 10.37, p < .01). These findings suggest that the effect of cocaine on withdrawal from methadone in infants is small, having either no contributory effect or only a mild degree of shortening of the duration of withdrawal symptoms.

ABNORMALITIES IN HEPATOCYTE ENDOCYTOSIS IN CHRONIC PANCREATITIS. Jaimie D. Nathan, Peter D. Zdankiewicz, Jinping Wang, Neal E. Seymour, John P. Geibel, Bhanu P. Jena, Dana K. Andersen. Departments of Surgery and Physiology, Yale University School of Medicine, New Haven, Connecticut.

Chronic pancreatitis (CP) is associated with impaired glucose tolerance, reduced hepatic sensitivity to insulin and a loss of insulin receptor (IR) availability on hepatocyte plasma membranes. The insulin-mediated reduction in the hepatocyte membrane-bound glucose transporter protein GLUT2 is impaired in CP as well. These abnormalities may be the result of altered intracellular vesicle trafficking. Hepatocyte fluid-phase endocytosis (FPE) was therefore assessed by in vivo uptake of FITC-Dextran (FITC-D) by confocal microscopy in livers from fed and fasting sham-operated (sham) rats and rats in which CP had been induced two to three months earlier by pancreatic duct oleic acid infusion. FITC-D uptake was greatly reduced in sham rats allowed access to chow ad libitum, compared to fasting rats (p < .001). In another experiment, 45 minutes after duodenal feeding in sham rats, FITC-D uptake remained low compared to fasting livers (p < 0.05). No significant inhibition of FITC-D uptake was seen in CP hepatocytes after either mode of feeding. To determine whether GLUT2 is actively internalized in hepatocytes and whether this receptor-mediated endocytosis (RME)-associated event is disordered in CP, livers were fractionated by sucrose density gradient ultracentrifugation to yield endosome (E)- and plasma membrane (PM)-enriched fractions, and GLUT2 content was quantified by Western blotting and scanning densitometry. Compared to fasting sham rats, the E:PM ratio of GLUT2 increased sharply in fed sham rats (p < 0.05). There was no change in the E:PM ratio of GLUT2 in CP livers following duodenal feeding. To test our findings using confocal microscopy, GLUT2 immunofluorescence was quantified by mean pixel intensity in an 8 x 16 pixel area of PM and a 16 x 16-pixel area of cytosol (CYT) in each of 30 random cells/field (400x) in each of three rats per group. Duodenal feeding increased the CYT:PM ratio of GLUT2, compared to fasting sham rats (p < .0001), while the CYT:PM ratio in CP remained unchanged. We conclude that following feeding, rat hepatocytes in vivo exhibit a reduction in FPE, and an increase in RME, which induces a shift in GLUT2 from the plasma membrane to the endosomal pool. Both the feeding-induced reduction in FPE and internalization of GLUT2 are lost in hepatocytes from CP rats, suggesting that the more central abnormality may be a failure of hepatocytes to convert from FPE to RME. Disordered regulation of endocytosis and impaired GLUT2 internalization may play a role in the glucose intolerance associated with CP.
STAIR-STEP ARTIFACT IN HELICAL-CT VIRTUAL COLONOSCOPY: PITCH AND ANGLE DEPENDENCE. Jason Scott Oliphant, James A. Brink and Elizabeth McFarland*. Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut and *Department of Radiology, Mallinckrodt Institute of Radiology, Washington University, St. Louis, Missouri.

This study was performed to characterize and quantify the imaging features of the stair-step artifact observed with helical-CT colonography. The effects of pitch, collimation and angle of the object plane were examined. An in vitro colon phantom comprised of an air-filled, smooth polyethylene tube was submerged in a water bath and scanned under helical CT protocol at orientations of 0, 30, 45 and 60 degrees relative to the z-axis. Three mm collimation, pitch of 1.0, and 5 mm collimation, pitch of 1.6, were used. Additional scans were performed at 45 degrees with pitches of 1.0 and 2.0 for both the 3 mm and 5 mm collimations. To minimize aliasing, transaxial images were reconstructed with at least 70 percent overlap. Analysis was perfomed on coronal reformations (2D) and volume render (3D VR) images. Line profiles were recorded across the artifacts. Stairstep and rippling (of alternating light and dark bands) artifacts were observed in the 2D and 3D reformations, respectively. The height of the stair-step and distance between the ripples (in the z-direction) were equal to half of the table increment. As the angle deviation increased, the artifact increased in transverse width on the 2D reformations and the bands increased in intensity on 3D reformations. In conclusion, the rippling artifacts seen in CT colonography were produced with properties that closely match previously documented interpolation artifacts with helical CT. Enhancement of the phasic properties is seen with 3D VR images.

DETECTION OF THE FACTOR V LEIDEN MUTATION IN A NONSELECTED BLACK POPULATION. Paul S. Pottinger, Fridbjorn Sigurdsson and Nancy Berliner. Section of Hematology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study was to determine the prevalence of the Factor V Leiden mutation among black and non-black inpatients and outpatients at the Yale-New Haven Hospital. We had found no previous information on the prevalence of this mutation within the black population, although it had been predicted by some that the abnormality might be found predominantly in individuals of European extraction. Randomly selected blood samples were obtained from the Yale-New Haven Hospital hematology laboratory and ethnic background was determined from hospital records. In addition, we studied stored DNA samples from black individuals already available in the laboratory from previous population studies. Using previously described methods, genomic DNA was isolated and analyzed by PCR and restriction enzyme digestion to identify the factor V cleavage site gene associated with activated Protein C (APC) resistance. Results were obtained on 214 black individuals, of whom three (1.4 percent) were heterozygous for the Factor V Leiden mutation. The incidence of the mutation in a similarly selected group of 126 non-black patients was 1.6 percent, yielding a relative risk of 0.88 (Fisher Exact two-tailed test, p = 1.0000, 95 percent confidence interval = 0.12 to 7.64). These results do not support the hypothesis of a difference between the prevalence of the Factor V Leiden mutation in the black and non-black populations studied.
BEDSIDE DOPPLER IDENTIFICATION OF LOWER-EXTREMITY DEEP-VEIN THROMBOSIS. Gregory S. Raskin and Robert C. Reiser. Section of Emergency Medicine, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut, and Department of Emergency Medicine, University of Virginia, School of Medicine, Charlottesville, Virginia.

This study compared the results of handheld Doppler ultrasound performed at the bedside with the results of formal Doppler ultrasound performed in the department of diagnostic imaging for evaluation of deep-vein thrombosis (DVT).

We carried out a prospective six-month study in an urban teaching hospital emergency department. Patients who were scheduled to undergo formal duplex ultrasound studies to rule out DVT underwent handheld Doppler ultrasound in the Emergency Department (ED) by an ED attending physician or medical student before the formal study, which was conducted in the department of diagnostic imaging. The radiologists were blinded to the results of the ED Doppler examination.

Unilateral duplex ultrasonography and handheld Doppler bedside examination were performed in 30 patients. Four patients were found to have proximal lower-extremity DVT on Doppler ultrasonography, and 26 were found to be free of DVT. Handheld Doppler ultrasound yielded three true-positive results, five false-positive results, 21 true-negative results and one false-negative result for a sensitivity of 75 percent, a specificity of 81 percent, a positive predictive value of 65 percent and a negative predictive value of 96 percent.

Handheld Doppler ultrasound examination in the ED is helpful in the evaluation of patients with suspected lower-extremity DVT. Further study is needed to identify the patients in which this type of examination is not reliable.

SUPERIOR MESENTERIC ARTERY FLOW VELOCITY WAVEFORMS IN SMALL-FOR-GESTATIONAL-AGE FETUSES. Eleanor H.J. Rhee, Laura Detti and Giancarlo Mari. Departments of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

The objective of this study was to analyze the superior mesenteric artery flow velocity waveforms in small-for-gestational-age fetuses, and to compare its contribution to the management of these fetuses with that already provided by the middle cerebral artery and umbilical artery flow velocity waveforms. Middle cerebral artery, umbilical artery and superior mesenteric artery flow velocity waveforms were prospectively obtained in 41 small-for-gestational-age fetuses with color Doppler ultrasonography. The pulsatility index was used to quantify the waveforms. Poor perinatal outcome was defined by fetal distress, perinatal death, need for assisted ventilation, and necrotizing enterocolitis. In the small-for-gestational-age fetuses, the middle cerebral artery pulsatility index was abnormal in 22/41; the umbilical artery in 26/41; and the superior mesenteric artery in 17/41. Coincident with abnormal umbilical and middle cerebral artery flow velocity waveforms were greater occurrences of poor perinatal outcome. The abnormality of an increased pulsatility index in the superior mesenteric artery velocity waveforms of small-for-gestational-age fetuses suggests greater vascular resistance and an overall reduction in visceral perfusion. However, the study of the superior mesenteric artery seemed only to support the information already provided for by the middle cerebral and umbilical arteries.
ELDERLY PATIENTS WITH CHRONIC ATRIAL FIBRILLATION: AN ASSESSMENT OF CARDIAC ATRIAL FIBRILLATION: AN ASSESSMENT OF CARDIAC STATUS AND FUNCTION. Bernice Ruo (Sponsored by Michael D. Ezekowitz). Section of Cardiology, Department of Internal Medicine, Yale University., School of Medicine, New Haven, Connecticut.

Atrial fibrillation is associated with increased morbidity and mortality. Because its prevalence increases with age, debates concerning management options are particularly important in the elderly. The options center around the use of cardioversion to restore sinus rhythm and the strategy of rate control with anticoagulation to reduce stroke risk. Central to this decision is the minimization of symptoms associated with chronic atrial fibrillation.

It is possible that the cardiac status and function in elderly patients with chronic atrial fibrillation will be inferior to those of age-matched controls in normal sinus rhythm. This study was designed as a case-control study to test this hypothesis. Holter monitors, echocardiograms and exercise stress tests were performed on 29 age-matched pairs (atrial fibrillation/sinus rhythm pair) to assess heart rate ranges, cardiac dimensions and exercise capacity. The past medical history of the two groups showed similar prevalences of hypertension, angina, valvular disease, pulmonary disease, diabetes and thyroid disease. However, the prevalence of congestive heart failure was significantly higher in patients with atrial fibrillation.

Holter monitoring showed that patients with atrial fibrillation have a wider heart rate range and longer pauses (RR intervals), but comparable mean heart rates and frequency of ventricular ectopy. Echocardiographically, patients with atrial fibrillation had significantly larger left atria and a higher prevalence of mitral regurgitation. However, their ejection fractions were comparable to controls. There was no significant difference in their exercise capacity as measured by duration of exercise with the modified Bruce protocol (9.5 ± 4.2 min. for patients with atrial fibrillation vs 10.2 ± 4.2 min for controls, p = .471).

The lack of difference in exercise capacity in elderly patients with chronic atrial fibrillation as compared with their age-matched controls suggests that rate control and anticoagulation may be a good choice for management of chronic atrial fibrillation in some elderly patients.

EFFECTS OF STRAIN ON HUMAN DERMAL FIBROBLASTS IN AN IN VITRO MODEL. Chrys Delling Schmults, Thomas L. McCarthy, Michael Centrella and Bauer E. Sumpio. Divisions of Plastic and Vascular Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.

We have developed an in vitro model that exposes human dermal fibroblasts (HDFs) to a constantly increasing strain and have evaluated the effects of this strain on cellular proliferation, morphology, alignment and production of collagen and non-collagen protein. Cells were plated on flexible wells coated with collagen I or fibronectin. A maximum strain of 24 to 25 percent was achieved over one to two day periods with an average strain of five to seven percent across the wells. Cellular proliferation was evaluated via Coulter counter. Morphology and alignment were evaluated by crystal violet staining and microscopic observation. Collagen I and III mRNA production was assessed with Northern blots. Collagen (CDP) and non-collagen protein (NCP) synthesis were measured via immunohistochemical studies and incorporation of 3H proline.

No differences were seen between strained and control cells in cellular morphology, alignment or collagen mRNA production. In one experiment, strained cells had a signific-
cantly lower cell number (P = 0.002). However, in other experiments, no differences in cellular proliferation were seen. In general, strained cells showed trends toward increased synthesis of CDP. However, only NCP synthesis was shown to significantly increase with strain. Significant increases in NCP were seen both in studies of protein synthesis over the entire experimental period (fibronectin wells: P = .04, collagen wells: P = .01) and in rate studies of protein produced over a two-hour period (P = .05). There was also a trend toward decreased CDP and NCP degradation with strain. A decreased rate of degradation may contribute to the increased amount of protein seen with strain. A two to five fold increase in CDP and NCP was noted in HDFs grown on fibronectin-coated wells as compared to collagen I-coated wells.

We have developed a new in vitro model for studying the effects of strain on cells. We have found trends toward increased collagen synthesis and decreased protein degradation in HDFs exposed to strain. Non-collagen protein synthesis is significantly elevated with strain in several experiments. We have also shown that HDFs grown on fibronectin surfaces produce more collagen and non-collagen protein than HDFs grown on collagen I surfaces. Further work remains to determine which strain conditions result in maximal changes in protein production in HDFs.

THE EFFECTS OF CHORDA TYMPANI NERVE TRANSECTION IN HUMANS.
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Fungiform papillae (the structures that contain taste buds on the anterior tongue) are innervated by two cranial nerves. The chorda tympani (VII) transmits taste information and the trigeminal (V) transmits somatosensory information (pain, touch and temperature). The chorda tympani fibers innervate receptor cells in the taste buds located on the apical epithelium of the fungiform papillae.

The chorda tympani nerve is frequently severed during otologic procedures where it passes through the middle ear, thus abolishing taste sensation on the ipsilateral anterior tongue. There have been numerous animal studies investigating the results of chorda tympani transection on the taste bud and fungiform papillae anatomy. Although there is species variation, these studies generally suggest degeneration of the taste structures with denervation. The anatomical association between trigeminal neurons and the taste buds raises the question of what the consequences of degeneration of the taste buds and the fungiform papillae might be for somatosensory perception in humans. Consequently, we studied a group of eight patients with unilateral transection of the chorda tympani nerve, peripheral to the geniculate ganglion.

Patients rated the perceived intensities of irritation/pain, touch, and thermal stimuli applied to the operated and non-operated (i.e., control) sides of the anterior tongue using the Labeled Magnitude Scale. Fungiform papillae were then counted by video microscopy and under direct visualization.

We confirmed a previously reported release of inhibition for taste stimuli at the contralateral circumvallate papillae in these patients. All irritation/pain and thermal sensations were significantly reduced on the operated side. Irritation/pain sensations tended to be the most profoundly reduced. In addition, we found no difference in the number of fungiform papillae on the operated versus control sides.
CLINICAL TRIALS AND OBSERVATIONAL STUDIES: HOW IMPORTANT IS RESEARCH DESIGN? Nirav R. Shah, John Concato and Ralph I. Horwitz. Medical Service, VA Medical Center, West Haven, Connecticut, and Department of Internal Medicine, Yale University School of Medicine New Haven, Connecticut.

Observational studies are often distrusted because of a concern that results may be vulnerable to many forms of bias, whereas randomized controlled trials have become the accepted method of evaluating efficacy in clinical medicine. In particular, observational studies are thought to overestimate the magnitude of exposure-outcome associations, based on examples from historical control trials. Our objective was to examine whether the type of research design systematically affects the point estimate of associations encountered in clinical research. We hypothesized that randomized trials would show less heterogeneity around a point estimate than observational studies, while results pooled within each type of study design would approximate the other.

We sought to compare published meta-analyses for clinical topics in which pooling was done using randomized trials and observational studies. Data were obtained by reviewing all meta-analyses published in five major journals during 1991-95, identifying topics for which both original randomized trials and observational studies were available. We then systematically evaluated the original trials for adherence to standards of methodological quality, using previously validated criteria for randomized trials, case-control studies and observational cohort studies. The pooled results for randomized trials and observational studies were then compared with regard to the point estimates and confidence intervals for associations of exposure and outcome.

Regardless of study design as randomized trials vs. observational studies, the pooled point estimates (and corresponding 95% confidence intervals) were similar for each of the five topics identified:

| Clinical topic                                      | Randomized Trials (95% C.I.) | Observational Studies (95% C.I.) |
|-----------------------------------------------------|------------------------------|----------------------------------|
| Efficacy of BCG against tuberculosis                | 0.49 (0.34 - 0.70)           | 0.50 (0.39 - 0.65)               |
| Mortality after screening mammography               | 0.79 (0.71 - 0.88)           | 0.61 (0.49 - 0.77)               |
| Cholesterol reduction and traumatic death           | 1.42 (0.94 - 2.15)           | 1.40 (1.14 - 1.66)               |
| Treatment of hypertension and risk of stroke        | 0.58 (0.50 - 0.67)           | 0.62 (0.60 - 0.65)               |
| Treatment of hypertension and risk of coronary heart disease | 0.86 (0.78 - 0.96) | 0.77 (0.75 - 0.80) |

In addition, results were similar for subgroups of studies that met a priori quality standards.

Our investigation suggests that observational studies do not systematically overestimate exposure-outcome associations relative to randomized controlled trials, and meta-analyses of observational studies should have a place along the spectrum of evidence that is used to guide clinical practice.
COMPLICATIONS OF CONJUNCTIVITIS DUE TO PSEUDOMONAS AERUGINOSA IN A NEWBORN INTENSIVE CARE UNIT. Samir S. Shah and Patrick G. Gallagher. Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

In infancy, Pseudomonas aeruginosa conjunctivitis may lead to a rapidly progressive invasive eye infection. In some cases, this destructive eye disease is associated with or followed by infection at other sites. We observed several hospitalized infants with P. aeruginosa conjunctivitis who developed systemic complications of P. aeruginosa infection without evidence of invasive eye disease, prompting us to examine the characteristics of this infection and its associated systemic complications in hospitalized infants. We retrospectively reviewed the course, treatment and outcome of infants with non-epidemic P. aeruginosa conjunctivitis in the Newborn Special Care Unit at Yale-New Haven Hospital over a 10-year period from November 1, 1986, to October 31, 1996.

Eighteen infants with P. aeruginosa conjunctivitis had mean birth weights and gestational ages of 29.3 weeks and 1380 g, respectively. The average postnatal age at onset of P. aeruginosa conjunctivitis was 17 days. No infant had invasive eye disease. Systemic complications occurred in seven (39 percent) infants and included bacteremia, meningitis, brain abscess and death. Infants who developed systemic complications had lower mean birth weights (850 g vs. 1716 g, p = .04) and lower mean gestational ages (26.4 vs. 31.2 weeks, p = .02) than infants who did not. Six of seven infants weighing <1000 g developed systemic complications; two of these infants died. In hospitalized infants, P. aeruginosa conjunctivitis may be associated with systemic complications with or without invasive eye infection, emphasizing the need for early detection and treatment of this infection in this population.

A FIVE-YEAR RETROSPECTIVE STUDY OF TUBERCULOSIS AT A PUBLIC HOSPITAL IN THE ECUADORIAN HIGHLANDS. Eric G. Shmookler, Peter A. Selwyn and Patrick O'Connor. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

This study focuses on the challenges facing tuberculosis (TB) control programs in developing nations such as Ecuador. METHODS: Through a retrospective chart review investigating a largely indigenous, Quechua-speaking population at a public hospital in rural Ecuador, we analyzed characteristics of all known TB cases and evaluated risk factors for non-completion of TB treatment in this population. RESULTS: Of the 192 patients in this study with active TB, 64.1 percent were male; the average patient age was 35.15 (± 18.52) years; 21.4 percent had a prior history of tuberculosis treatment; the average distance between patient residence and the hospital was 9.9 (± 11.5) kilometers; and 57.9 percent of patients lived in the mountains above 3000 meters. 42.2 percent of patients failed to complete treatment. Statistical analysis showed the following to be significant risk factors for non-completion of treatment: a) prior treatment for tuberculosis, (OR 3.03; p = 0.032); b) increased patient age (p = 0.035); c) living over 3000 meters, (OR 1.96; p = 0.031); and d) increased distance between patient residence and the hospital (p = 0.0007). Neither patient gender nor disease site (pulmonary versus extrapulmonary) were significant risk factors. CONCLUSIONS: Developing nations, such as Ecuador, face unique public health problems related to limited infrastructure, difficult terrain, poverty, and lack of education regarding tuberculosis. Efforts designed specifically for this environment must be employed to improve identification and effective treatment of infectious patients. Such efforts could include improved clinical evaluation of symptomatic patients,
consistent use of sputum analysis in diagnosis, incorporation of cultural, linguistic, organizational, and pharmacological changes to make treatment more successful and accessible, use of incentives to keep patients in treatment, and improved outreach programs which take advantage of the existing local infrastructure to evaluate contacts of infectious patients, locate patients who abandoned treatment, and, ideally, establish an effective program of directly observed therapy.

THE PROCESS OF MODERN MAMMOGRAPHY: EXPLORING HIDDEN COSTS AND SELF-REFERRAL. Lisa Gale Suter and Joann G. Elmore. Section of General Internal Medicine, Department of Medicine and Epidemiology, University of Washington School of Medicine, Seattle, Washington. (Sponsored by Janet Henrich, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut).

Research has yet to explore the impact of hidden costs, such as transportation, childcare and parking, on mammography screening in the United States. Mammography is unique among radiological tests in that it can be obtained without a physician's referral, but the rate of self-referral for mammography is unclear. Therefore, we sought to quantify and better describe hidden costs and self-referral in mammography.

A questionnaire assessing characteristics such as respondent age, costs associated with obtaining a mammogram (including direct and indirect costs and hidden costs) and source of referral was given to all women obtaining mammograms at nine Connecticut mammography facilities during a two-week period. Hidden costs included lost work time, parking and childcare costs. Responses from women seen recently by their primary health care provider were compared to self-referred women, defined as those who had not seen a health care provider in the year prior to the mammogram.

The response rate was 62 percent (731/1189), and the mean age of respondents was 58 years (range, 30-100 years). Thirty-four percent of respondents reported incurring some type of hidden cost, and 23 percent thought cost might prevent them from obtaining a future mammogram. Self-referred women constituted 16 percent of respondents and were more likely than provider-referred women to use mobile facilities (70 percent vs. 36 percent, p < .01), to lack insurance coverage for mammography (14 percent vs. 6 percent, p = .02), to be knowledgeable of their out-of-pocket cost for their mammogram (84 percent vs. 74 percent, p = .02) and to report cost as a deterrent to obtaining a future mammogram (36 percent vs. 21 percent, p < .01).

Hidden costs are significant and may impact screening behavior. Many women obtain mammograms without the guidance of a primary care provider, especially at mobile mammography facilities. Further study is needed to assess the clinical impact of these issues on mass screening programs.

SMOKING BEHAVIOR AND HEALTH BELIEFS IN ADOLESCENTS WITH ASTHMA. Amy E. Taylor. (Sponsored by Walter R. Aryan). Section of Adolescent Medicine, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

The purpose of this study is to compare the prevalence of cigarette smoking and the beliefs about tobacco use in adolescents with asthma to those without respiratory disease. A total of 103 adolescents were surveyed, ranging in age from 13 to 17 years, using a written questionnaire and a brief verbal interview. Subjects were recruited from the
Adolescent Clinic of the Yale Primary Care Center, the Pediatric Pulmonary Clinic and the New Haven Detention Center. Results were analyzed using adjusted odds ratio, chi-square and T-tests. We found a smoking prevalence of 40 percent, and overall, asthmatics and nonasthmatics had similar prevalences of smoking. However, when the severity of asthma was taken into account, we found that moderate/severe asthmatics had a smoking prevalence of 25 percent, compared to 48 percent of those with mild asthma. The participants' knowledge about the health risks of tobacco use was examined using a series of questions that were graded on a five-point scale, with a higher score reflecting more awareness of the dangers of smoking, and a more negative opinion of tobacco use overall. We found no significant difference in the health belief scores between either of the asthmatic groups and the nonasthmatics. Another interesting result was the similarity of scores between the smokers and the nonsmokers. We conclude that, in contrast to our hypothesis, asthmatic teenagers are not better educated about the risks of smoking and are not more likely to be nonsmokers. In addition, the lack of correlation between smoking status and health belief score, indicates that simply providing adolescents with facts does not deter them from smoking.

IRON SUPPLEMENTATION AND THE APPROACH TO IRON DEFICIENCY ANEMIA IN HOSPITALIZED PATIENTS. Sushrut S. Waikar and Peter McPhedran. Departments of Laboratory Medicine and Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Iron supplementation is commonly prescribed in the inpatient setting. We evaluated the iron prescribing practices of physicians at Yale-New Haven Hospital and the approach to iron deficiency anemia among inpatients treated with iron. Two groups of patients were prospectively identified by reviewing daily inpatient pharmacy logs of iron prescriptions. The first group consisted of consecutive inpatients on iron and provided information on hospital-wide practice of iron supplementation. The second group consisted of selected medical inpatients on iron and was used to assess the diagnosis and evaluation of iron deficiency anemia.

Fifty-two percent of all inpatients who received iron were post-operative; 26 percent were pregnant or post-partum; and 19 percent were patients otherwise suspected of iron deficiency. Three-quarters of all iron prescriptions came from surgical services. Among medical inpatients treated with iron, the majority (61 percent) had ferritin levels of more than 100 ng/dL, making them unlikely to be iron deficient. Iron deficiency accounted for anemia in only 23 percent of patients treated with iron. Other causes included anemia of chronic disease (29 percent) and renal insufficiency (28 percent). Housestaff used serum iron and total iron binding capacity (TIBC) more often than ferritin (76 percent vs. 62 percent) before deciding to prescribe iron; the test results were frequently misinterpreted. Comparing several lab tests, TIBC was the best test other than ferritin for iron deficiency. One-quarter of patients begun on iron were not tested for fecal occult blood. Patients who were iron deficient for the most part received appropriate gastrointestinal evaluations. Patients who were inappropriately judged to be iron deficient and started on iron supplementation did not undergo unnecessary gastrointestinal evaluations during hospitalization.

In summary, iron treatment is widely used among inpatients, most often for conditions that are not iron responsive. The laboratory diagnosis of iron stores is well studied in the literature but not well executed in practice, leading to inappropriate prescriptions of iron. Errors in diagnosis were fortunately not matched by errors in selection of patients for gastrointestinal evaluations.