

**Add to the Body of Knowledge about Normal and  
Abnormal Biological Functions and Behavior**

**SCIENCE ADVANCES**

- \$ Basic Research Shows How Folic Acid Protects Against Heart Disease, Birth Defects
- \$ Enzyme Can Repair Alzheimer's Tangles
- \$ Scientists Discover How Immature Cells Decide to Become a Liver
- \$ Heavy Metal Research is Music to Biologists' Ears
- \$ AIDS Enzyme Caught in the Act
- \$ Gene Involved in Anesthesia Response Identified
- \$ Fruit Fly Research Links Cocaine Sensitization With Biological Clocks
- \$ Potassium Channel Research May Shed Light on Heart Ailment
- \$ Inhibiting New Blood Vessel Growth Reduces Atherosclerotic Plaque in Mice
- \$ Combination of Therapies May Reduce Risk of Coronary Heart Disease in Women
- \$ Diet High in Fatty Fish May Protect Against Sudden Cardiac Death
- \$ New Understanding of Increased Risk of Blood Clots in Old Age
- \$ Insight into How Hepatitis C Virus Evades Host Defenses May Lead to New Therapies
- \$ Molecular Basis for Malaria Resistance and Susceptibility in Pregnant Women Defined
- \$ Molecular Studies of Hepatitis C Virus Identify Sequences that May Be Targets for Preventive Agents
- \$ Maternal HIV Blood Levels Are Strong Predictors for Risk of Perinatal Transmission
- \$ Latent Infection of CD4+ T Cells Provides a Mechanism for Lifelong Persistence of HIV-1
- \$ Identification of Salivary Anti-Thrombin from the Anopheles Mosquito May Lead to the Development of Novel Malaria Pharmaceuticals
- \$ Knowledge about an Immune Cell Receptor Type Provides Clues about Immunoregulatory Processes that Affect Pregnancy
- \$ Natural Model of Cutaneous Leishmaniasis Provides Insights into Vector's Role in Pathogenesis and Potential for Novel Prevention Strategies
- \$ Sequencing of Organism involved in Sexually Transmitted Diseases and Blindness Provides Clues for Vaccine and Drug Development
- \$ Preventing Unwanted T Cell Activation by Triggering CTLA-4, A Negative-Signaling Molecule
- \$ The Adult Thymus Is Capable of Rebuilding the Immune System after Treatment of HIV Infection
- \$ Insulin Resistance in Tissues Underlies Type 2 Diabetes
- \$ Diabetic Heart Disease Still a Disproportionate Problem
- \$ Increased Insulin Sensitivity and Obesity Resistance in Mice
- \$ Paracellin-1, a Renal Protein, is Required for Magnesium Homeostasis
- \$ Types of Pituitary Gland Cells: How Are They Determined?
- \$ New Mechanisms of Resistance to Urinary Tract Infection are Identified
- \$ Understanding the Regulation of Gene Expression
- \$ Pathways In the Brain That Control Food Intake
- \$ Turning Brain into Blood // Turning Bone Marrow into Liver Cells
- \$ Mechanisms of Female Sex Determination and Development
- \$ New Therapeutic Approach for Inborn Metabolic Errors
- \$ The Role of Breast Cancer Gene (BRCA 1) Mutation in the Development of Breast Cancer
- \$ Progress in Zebrafish Research
- \$ Regulation of Genetic Expression During Blood Formation
- \$ Proteins as Genetic Material in Human Diseases
- \$ Appetite-Regulating Protein Implicated in Alcohol Consumption

- \$ Adolescents May Be Vulnerable to Some Types of Alcohol-Induced Memory Impairment
- \$ Scientists Discover Gene Mutations Defining New Group of Inflammatory Diseases
- \$ Inbred Mouse Strains Yield Clues to the Genetics of Bone Density
- \$ Febrile Seizures Modify Brain Excitability
- \$ Understanding the Potential of Neural Stem Cells
- \$ Gene for Narcolepsy Discovered
- \$ Understanding a Retinal Degenerative Disease
- \$ Retina-specific Gene Causes Autosomal Dominant Retinitis Pigmentosa
- \$ New Findings Link Nitric Oxide to Nerve Cell Damage in Glaucoma
- \$ New Clues to How the Lens Forms and Maintains Its Structure
- \$ Growth Factor Research May Lead to New Treatments
- \$ Identification of Modified Forms of  $\alpha$ B-Crystallin in Human Cataracts
- \$ The Dual Role of Interleukin-12 in Regulation of Autoimmune Retinal Disease
- \$ Interferon- $\gamma$  Increases the Severity of Uveitis and Induces Retinal Degenerative Changes in Transgenic Rats
- \$ Hair Cell Differentiation and Specification
- \$ A Novel Calcium Response in Hair Cells
- \$ Identification of Molecular Mechanisms of Pathogenesis of Otitis Media
- \$ The Vestibular System Influences Cardiovascular Control in Humans
- \$ Taste Receptors
- \$ Deciphering the Code for Odors
- \$ Pheromone Pathways of the Brain
- \$ Protein Has Potential to Treat Brain Damage
- \$ Preventing Early Miscarriage
- \$ Improving Treatment for Polycystic Ovary Syndrome
- \$ Islet-Specific Transcription Factors and Development of the Endocrine Pancreas
- \$ Effects of Estrogen on the Brain After Menopause
- \$ Identifying a Risk Factor for a Common Birth Defect
- \$ Killer Cells and Resistance to Cancer Metastasis
- \$ Gender Differences in Heart Muscle Function
- \$ Complement System May Be Useful Target for the Treatment of Sepsis
- \$ Substance Abuse Treatment Can Be Cost-effective
- \$ Chemical Identified That Can Block Brain Damage Caused By Methamphetamine
- \$ A Chemical Produced in the Brain May Offer New Insights into Tourette's Syndrome and Parkinson's Disease
- \$ Chronic Marijuana Smokers May Undergo Withdrawal When They Quit
- \$ Dopamine: More than Just the Pleasure Molecule
- \$ Health Effects of Cigar Smoking
- \$ Creating Human Cancer Cells
- \$ Published Research Using Linked SEER-Medicare Data
- \$ Interpretation of Emerging Patterns and Trends in Cancer
- \$ Gene For Making Mice Smarter Offers Clue To Human Intelligence
- \$ Clues to How our Brains Organize Visual Perceptions
- \$ New Brain Cells Formed In Response To Learning
- \$ How the Brain Pays Attention
- \$ Newly Identified Protein Essential For Message Transmission In The Brain
- \$ Clues to the Nature of Schizophrenia
- \$ New Players in the Molecular Basis of Memory and Learning
- \$ Learning How We Learn
- \$ Neural Activity Shapes the Brain's Cells

- \$ Depressed Mothers= Speech Affects Learning In Babies
- \$ Link Established Between Cessation of Cell Divisions and the Mammalian Aging Process
- \$ Caloric Restriction Slows the Aging Process
- \$ Gene Therapy Can Maintain Muscle Mass and Strength
- \$ Does the Relationship between Health and Economic Status Reverse Over the Life Course?
- \$ Protein Complexes in Cells Can Use Energy to Promote Subsequent Function or Loss of Function
- \$ Control of Programmed Cell Death in Human Tumor and Immune Cells
- \$ Gene on Chromosome 13 Linked to a Form of Familial Early-Onset Dementia
- \$ Two Amino Acid Residues are Critical to Presenilin Protein Activity: Leads to Potential Targets for Treatment of Early-Onset Alzheimer's Disease
- \$ New Neurons Are Produced in the Adult Human Brain
- \$ Age Does Not Change the Ticking of the Circadian Clock, but it's Faster than We Thought
- \$ Synchrotron Resources Enable Landmark Studies of Ribosome Structure
- \$ HIV Infection Persists Even With Combination Drug Therapy
- \$ Nuclear Magnetic Resonance Reveals More Pieces of the Prion Puzzle
- \$ AIDS Virus Strains in Africa: Novel and More Virulent
- \$ Cooking Fuel, Indoor Pollution and Tuberculosis in India
- \$ Lung Disease in Rice Granary Workers
- \$ Gene Therapy Restores Muscle in Aging Mice
- \$ Nitric Oxide Perfusion in Patients with Sickle Cell Disease
- \$ Evidence that Alcohol Has Adocking Sites@ on Cells Raises Potential for New Medications
- \$ Alcohol Consumption Influenced by Different Genes in Females and Males, Suggesting that They May Process Alcohol Differently
- \$ Skeletal Muscle Damage Induces Heart Disease
- \$ Tumor Necrosis Factor Mediates Orthopaedic Implant Osteolysis
- \$ Molecular Basis of the Physical Connection Between Epidermis and Dermis
- \$ Hair: Molecular Biology, Embryology, Cycling and Diseases
- \$ Growth Factors Prevent Loss of Embryonic Nerve Cells Exposed to Toxins in Test Tube
- \$ Violence Reduction Sustained After Alcoholics Receive Behavioral Marital Therapy
- \$ Light-to-Moderate Drinkers Account for More On-the-Job Problems than Do Heavy Drinkers
- \$ One In Four U.S. Children Exposed to Alcohol Abuse or Alcoholism in Family
- \$ Newly Discovered Genes May Contribute to Epilepsy
- \$ Does Moderate Alcohol Intake Protect the Heart? Scientists Track Pathways that Could Lead to Cardioprotection
- \$ Steroid-Induced Bone Loss: Mouse Findings Point to Preventive Possibilities
- \$ Risk of Hip and Wrist Fractures Shown Linked to Chromosome 19 Gene
- \$ Physical Activity and Osteoarthritis
- \$ Restoring Production of Brain Cells in Old Age
- \$ Killing of Intracellular *Mycobacterium tuberculosis* by an Antimicrobial Protein Found in Human Cytotoxic T Lymphocytes
- \$ Ancient Receptors Trigger Inborn Reactions to Bacteria
- \$ Vigorous Cytotoxic Response Leads Recovery against Hepatitis C Virus
- \$ The Mammalian Gene Collection: A Resource for Studying Gene Expression and Function
- \$ Genetic Defect in Myeloid Leukemia Explained
- \$ Absence of Linkage between Bone Formation and Bone Loss
- \$ Mechanism of Fungal Adhesion Identified
- \$ Why Prostate Cancer Homes to Bone
- \$ Major New Tumor Suppressor Provides Fresh Insights into Cancer

- \$ Secretory Leukocyte Protease Inhibitor (SLPI) Inhibits Arthritis
- \$ Acceleration of Wound Healing in Aged Humans
- \$ Breast Cancer, Heart Disease, Osteoporosis and the  $\beta$ ERKO Mouse
- \$ A Common Link in Failed Pregnancies
- \$ Breast Cancer Susceptibility Gene, BRCA1 - How Does It Work?
- \$ Inhibitors of Growth Factors Inhibit Pulmonary Fibrosis
- \$ Subtle Mutations Can Have Disastrous Effects When Combined
- \$ Signaling Environmental Stress B How Do Cells Respond to Their Surroundings?
- \$ Chemokines and Multiple Sclerosis

### **SCIENCE CAPSULES** (page 157)

- \$ Anchoring Fibrils
- \$ Lupus Diagnosis and Survival
- \$ Genetic Studies of Systemic Lupus Erythematosus (SLE)
- \$ Friedreich-s Ataxia
- \$ The Brain-s Capacity to Change
- \$ A New Approach to Recovery After Spinal Cord Injury
- \$ Imaging Pain in Humans
- \$ Structural Basis of Multidrug Recognition Determined
- \$ Structure of Key Component in Programmed Cell Death Determined
- \$ Understanding Clathrin Mediated Endocytosis
- \$ New Explanation for Why Lactic Acid in Bloodstream Rises During Severe Injury, Sepsis, and Heart Failure
- \$ Crystal Structure of Enzyme That Produces Key Cell Molecule Determined
- \$ Molecular Basis of Spinal Muscular Atrophy Discovered
- \$ Broader Role of Telomerase in Extending Cell Life
- \$ Induction of Tolerance to Antigens
- \$ Genetic Determination of Type 1 Diabetes Autoimmunity
- \$ Intensive Therapy of Type 1 Diabetes Reduces Collagen-Based Complications
- \$ Prediction of Coronary Disease in Type 1 Diabetes
- \$ A Mathematical Model of Colonization by *H. pylori*, Pathogen in the Stomach
- \$ Cholesterol-rich regions in membranes are key for Stress Effects on Blood vesssel lining cells
- \$ The Gene for Recessive Polycystic Kidney Disease is Mapped
- \$ Identification of a Membrane Protein That Protects Against the Inflammation of Glomerulonephritis
- \$ Diamond-Blackfan Anemia: The First Human Disease Caused by a Mutation in a Gene for a Ribosomal Protein
- \$ The Immunosuppressant Cyclosporine Causes Cancer Progression
- \$ A Cloned Zebrafish Gene Is A Model for Human Congenital Anemia
- \$ A Red Blood Cell Membrane Protein is Important for Normal Membrane Integrity
- \$ Role of Niemann-Pick C1 Protein In Disease
- \$ Is a Low Leptin Concentration the Expression of the "Thrifty Genotype ?"
- \$ It Caught My Eye
- \$ Understanding Cataract Formation
- \$ Specificity in Visual Signaling Pathways
- \$ Organization of Neurons in the Cerebral Cortex
- \$ Analysis of Visual Motion
- \$ Rapid Visual Guidance of Movement
- \$ Mathematical Modeling of Rapid Eye Movements

- \$ Attentional Activity in the Cerebral Cortex
- \$ Pregnancy and Autoimmune Retinal Disease
- \$ Hereditary Factors Affecting Predisposition to Uveitis
- \$ New Insights into Hereditary Eye Tumors
- \$ Novel Roles of GDF-9 in Regulating Fertility
- \$ Understanding Early Miscarriage
- \$ Environment and IQ Level
- \$ The Role of Hormones in Maternal Behavior
- \$ Healing Damaged Spinal Cords
- \$ A Mouse Model of Host Defense Against Lung Inflammation
- \$ New Ways to Reduce Jet Lag on the Horizon
- \$ Genes That Control Early Brain Development
- \$ Powerful Anti-AIDS Agent Found in Tears and Urine of Pregnant Women
- \$ Maternal Caffeine Use and the Outcome of Pregnancy
- \$ A Better Way for Nurses to Assess Infant Pain
- \$ Clue to Excess Prevalence of High Blood Pressure in Blacks
- \$ A New Lesson From Sleeping Dogs
- \$ Inflammatory Findings on Diabetes
- \$ Homocysteine: Another Kind of Heart Risk
- \$ Too Much Ado About Mitral-Valve Prolapse
- \$ New Insights into Human Cell Aging
- \$ Declining Seroprevalence in New York City HIV Epidemic
- \$ Changing Drug Use Patterns
- \$ Who-s Using Methamphetamine? New Insight
- \$ The Environment of the Mouse Matters
- \$ Unraveling the Mysteries of Relapse
- \$ Further Evidence of the Association between Human Papillomavirus Infection and Cervical Cancer
- \$ Breast Cancer Genetics
- \$ Mechanisms of Angiogenesis
- \$ Functions of BRCA1
- \$ Identification of a Protein that Helps Maintain Genomic Stability
- \$ Prostate Cancer Genetics
- \$ Function of the Met Gene in Hereditary Papillary Renal Carcinoma
- \$ Hormone Levels, Dietary Soy, and Breast Cancer
- \$ What-s the Natural Purpose of a Marijuana Receptor?
- \$ The Way We Were: Making Vs. Storing Long-term Memories
- \$ Genetic Locus for Specific Language and Reading Deficits
- \$ Competition Among Brain Chemicals Suggests New Path to Medication Development
- \$ To Sleep, Perchance, To Have a Memory Work-out
- \$ Generational Transmission of Psychopathology
- \$ Delaying Menopause-Related Health Problems: Estrogen Protection With or Without Fertility
- \$ Identified Protein May Lead to Gene Therapy for Huntington-s Disease
- \$ Neighborhood and Socioeconomic Characteristics Make It Difficult to Initiate and Maintain Recommended Physical Activity Levels
- \$ Centenarians Live Most of Their Lives in Good Health
- \$ Chronic Inflammation in the Elderly May Lead to Disability and Early Death
- \$ Researchers Identify Genetic Mechanisms Involved in the Age-Related Increase of a Blood Clotting Factor

- \$ Scientists Gain New Insights into Pathways Controlling Immune Function
- \$ Common Mechanism Is Identified for Gene-Specific and X Chromosome Inactivation
- \$ One Form of the ApoE Gene Protects Brain Cells from Injury
- \$ Mutations in the APP Gene Inhibit Normal Protective Functions of the APP Protein
- \$ Can Tau Mutations Cause  $\beta$ -amyloid Deposition?
- \$ Gene Controlling Life-Span Identified
- \$ Lorenzo's Oil Prevents Neurodegeneration in a Fly Model of Human Disease
- \$ New Gene Causing Dementia Discovered
- \$ Mammalian Clock Genes
- \$ Inhibition of Inappropriate Thoughts and Impulses
- \$ Cytomegalovirus Accelerates AIDS Progression in Infants
- \$ Human Myostatin Gene Expression Contributes to Muscle Wasting in HIV-Infected Men
- \$ The Eyes Have It
- \$ Effects of Low Level PCB Exposure on Male Reproduction
- \$ Chimp Origin of HIV Found
- \$ Weakened Virus Still Causes Disease in Primates
- \$ Routine Maternal Use of AZT is Safe for Children
- \$ Cellular Immunity May Not be Necessary for HIV/AIDS Vaccine
- \$ Possible Relief for Allergy Sufferers
- \$ Key Immune System Enzymes Essential to Health
- \$ Mast Cells Protect Against Bacterial Infection
- \$ Progress in Developing an RSV Vaccine
- \$ Powerful Skin Toxin Identified
- \$ Protein Signals May Be Linked to Leukemia
- \$ Isolation of a Potential Activator of Latent Herpes Simplex Virus
- \$ Sequence of Chromosome 1 of *Leishmania major*
- \$ Potential Candidate for Herpes Simplex Virus Vaccine
- \$ Disease Promoting Enzyme Targeted
- \$ Infection May Be Risk Factor for Cardiovascular Diseases
- \$ Psychosocial Implications of XSCID
- \$ Isolation and Characterization of Human Cementoblasts
- \$ Mutations in GNAS1 Cause Fibrous Dysplasia of Bone
- \$ HistatinsB Promising Antifungal Agents
- \$ New Insights into Cartilage Development
- \$ Molecular Mechanism for Cleft Palate
- \$ Candidate Taste Genes Identified
- \$ Cartilage to Bone: The Role of Vascular Endothelial Growth Factor (VEGF)
- \$ Degradation of Oxidatively Damaged Histones Occurs Through Poly-ADP Ribose-activated 20S Proteasome
- \$ Novel Ubiquitin Chains in DNA Repair
- \$ Apoptosis in the Absence of Caspase Activity
- \$ Report on the Health Effects of Electrical and Magnetic Fields
- \$ Cloning of a Novel Kidney Cytochrome P450 Enzyme that Metabolizes Fatty Acids

**STORIES OF DISCOVERY** (page 199)

- \$ Drug Exposed Children: What the Science Shows
- \$ Neurobiology of Addiction: The Role of Dopamine
- \$ The Link Between Oral Biofilm Infections and Systemic Disease

*FY99 NIH GPRA Research Program Outcomes*

- \$ Friedreich's Ataxia and Molecular Mechanisms of Iron Transport
- \$ Challenging Obesity
- \$ Sun and Skin
- \$ Fetal Alcohol Syndrome
- \$ Building HIV/AIDS Research Capacity in Uganda
- \$ A Simple Vision Plan
- \$ An Appetite for Alcohol
- \$ Helping Couples Conceive
- \$ Progress in Understanding Alzheimer's Disease



## **Basic Research Shows How Folic Acid Protects Against Heart Disease, Birth Defects**

*Background:* Scientists have known for decades that folic acid, one of the B vitamins, can protect against certain birth defects--such as spina bifida--that develop in the first few weeks after conception. For this reason, the Food and Drug Administration (FDA) recommends that every woman of child-bearing age supplement her diet with 400 extra micrograms of this vitamin. Folic acid supplements may also decrease the incidence of heart attacks and strokes in both men and women. More recent findings have suggested the way in which folic acid does its molecular good deeds, by lowering levels of a potentially harmful compound called homocysteine, a risk factor for heart attacks and strokes.

*Advance:* New basic research shows that folic acid performs this task by speeding up the conversion of homocysteine to an amino acid, called methionine, that the body needs to fuel myriad chemical reactions. Folic acid saves the day, the researchers show, by improving the fit between an enzyme and a helper molecule called a cofactor. The enzyme in this case is methylenetetrahydrofolate reductase (MTHFR), and the cofactor, a molecule called FAD, is also vitamin-derived (from vitamin B2) and is essential for converting homocysteine to methionine. A team of scientists who were studying how the MTHFR enzyme works determined that folic acid performs its protective role in the body by locking FAD onto MTHFR.

*Implications:* Besides bolstering the FDA guidelines on the importance of dietary folic acid for pregnant women, the new results provide solid evidence for the molecule's important and more general role in reducing homocysteine levels that are dangerously high in a variety of unhealthy states, such as heart disease. The work also points to possible therapies for these diseases, by upping folic acid intake in the diet, either through foods rich in the vitamin or vitamin pills. [secondary B treatment]

Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, and Ludwig ML: The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. Nature Structural Biology 6:359-65, 1999.

## Enzyme Can Repair Alzheimer's Tangles

*Background:* Alzheimer's disease is the most common cause of dementia in older people, affecting an estimated 4 million people nationwide. As the disease progresses, nerve cells and the connections between them are gradually lost, which disrupts key brain functions. Hallmarks of the brains of people with Alzheimer's disease are abnormal tissue structures called plaques and tangles. Scientists have linked the appearance of tangles with memory loss and other symptoms of dementia. Researchers suspect that the tangles--basically knots of a long, stringy protein called "tau"--critically compromise the normal functioning of nerve cells by crippling their ability to communicate with neighboring cells. Normally, tau's job is to both assemble and maintain the cell's scaffolding apparatus (groups of proteins called microtubules) that stretch from one end of a nerve cell to another and ferry nutrients and structural components. Tau gets into trouble and cannot keep its proper structure, scientists believe, when it acquires too many molecules of a chemical tag called a phosphate.

*Advance:* This year, basic researchers studying the process of cell division identified an enzyme that, in a test tube, can coax the phosphate-laden tau jumble back to its normal shape. The enzyme, dubbed Pin1, somehow rehabilitates tau proteins so that they in turn can properly assemble microtubules side by side into the threadlike shape that forms the cellular scaffold. Interestingly, tissue inside the brains of people with Alzheimer's disease is conspicuously short of available Pin1, because Pin1 is apparently depleted by working overtime to keep fixing tau. These results lead the researchers to believe that this critical enzyme somehow is unavailable to repair tangles as they form.

*Implications:* Pin1's detangling job is not limited to the brains of people with Alzheimer's disease. The enzyme also juggles phosphate-tagged proteins that regulate the timing of cell division in many different cell types. The discovery of the healing power of Pin1 in nerve tissue may aid in researchers' quest for new therapies to treat Alzheimer's disease and other so-called neurodegenerative diseases that disable the brain. [secondary B treatment]

Lu P-J, Wulf G, Zhou XZ, Davies P, and Lu KP: The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. Nature 399:784-8, 1999.

### **Scientists Discover How Immature Cells Decide to Become a Liver**

*Background:* Organs are complicated collections of cells that together perform important functions in the body. The liver, for example, plays a key role in processing and detoxifying foreign substances, which include everything from helpful medicines to dangerous poisons. Helping to control levels of blood sugar is yet another of the liver's important functions. Thus, the entire body is placed at serious risk when this organ becomes disabled by diseases such as hepatitis, alcoholism, or cancer. Scientists yearn to be able to decipher the master set of instructions that tells immature cells in the embryo how and when to mature and form one organ, but not another.

*Advance:* In an important first step toward unraveling some of these secrets, scientists have discovered a class of related proteins that commit embryonic precursor cells to mature into liver cells. New studies show that at least three of these molecular signals, which are fibroblast growth factors called FGF1, FGF2, and FGF8, direct precursor cells that could become part of the lung, stomach, liver, thyroid, or pancreas to commit to becoming a liver cell. Cells in all of these organs share a common ancestor called a definitive endoderm cell, which itself traces back to the few cells that constitute the days-old embryo.

*Implications:* Understanding the body's decision-making process when it comes to forming organs from embryonic cells has implications for tissue regeneration--the process of rebuilding a diseased or damaged organ or tissue, or further into the future, for making new organs or tissues from scratch. Knowing how the body flips such genetic "switches" as the FGF signaling pathways will also aid scientists in understanding the origin of diseases that strike the liver as well as in developing means to treat those ailments. [secondary B treatment]

Jung J, Zheng M, Goldfarb M, and Zaret K: Initiation of mammalian liver development from endoderm by fibroblast growth factors. Science 284:1998-2003, 1999.

### **Heavy Metal Research is Music to Biologists' Ears**

*Background:* Crab and lobster are more than perennial summer favorites--they are also a good dietary source of copper. Yes, the stuff of pennies is crucial for life--the metal copper is an important helper to many cellular enzymes, including superoxide dismutase (SOD), which sops up dangerous "free radicals" that accumulate inside cells. Defects in SOD have been linked to some inherited forms of amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. Although copper is necessary for life, it is a potentially toxic "heavy metal" that--in the wrong cellular locale--can damage other molecules, and in some cases can even cause disease.

*Advance:* In an important advance in understanding the molecular underpinnings of ALS, basic researchers have deciphered the three-dimensional structure of a yeast copper "chaperone" protein. This protein transports copper to the SOD enzyme and protects copper from unwanted cellular interactions along the way. In order to "see" the chaperone protein, the researchers used a technique called X-ray crystallography. In this technique, scientists bombard a tiny crystal of protein with high-energy X-rays, then piece together the protein's shape by tracing the directions in which the energy is scattered. Knowing what a protein looks like can say a lot about how it works.

*Implications:* Determining the structure of the copper chaperone protein offers scientists new insights into the way it might interact with SOD. In ALS, researchers suspect, the defective SOD--when energized with the copper it needs to function--runs amuck and causes cellular damage. With the new knowledge in hand, the scientists predict that they will soon be able to catch the chaperone-SOD duo in the act of trading off copper, which offers drug developers a crystal-clear glimpse of how to cripple this molecular embrace in patients with Lou Gehrig's disease. [secondary B treatment]

Lamb AL, Wernimont AK, Pufahl RA, O'Halloran TV, and Rosenzweig AC: Crystal structure of the copper chaperone for superoxide dismutase. Nature Structural Biology 6:724-9, 1999.

## AIDS Enzyme Caught in the Act

*Background:* AZT and many other anti-AIDS drugs target an enzyme in the human immunodeficiency virus (HIV) known as reverse transcriptase. This enzyme translates the virus' genetic material into a form that can insert itself into human chromosomes. The virus then commandeers the infected cell, forcing it to produce new virus particles.

AZT works by stopping reverse transcriptase's translation mid-word. But some, slightly altered versions of the enzyme--which are present in almost every person with HIV--can evade AZT's effects. Virus particles containing these forms of the enzyme multiply, and eventually, AZT is no longer effective against the virus. Everyone receiving AZT alone develops such resistance to it--and can spread the resistant virus to others.

*Advance:* Scientists have been trying for years to determine the structure of reverse transcriptase in its active form. These researchers succeeded by using an innovative chemical technique to tether the enzyme to its natural biochemical partners.

The detailed, three-dimensional snapshot reveals in atomic detail how the enzyme foils AZT, and reveals a possible target for new anti-AIDS therapies.

Reverse transcriptase is shaped something like a relaxed hand. The new structure reveals that, as the enzyme translates viral genetic material from RNA into DNA, the "hand" clenches around the growing DNA strand. AZT stops the process by capping the growing end of the DNA strand. The structure confirmed what many scientists had suspected for years--that most of the mutations that make the enzyme resistant to AZT cluster around the capping site, where the drug binds to DNA.

*Implications:* The new information reveals, at a molecular level, how HIV becomes resistant to AZT-like drugs. It also provides detailed insight into how to develop better anti-AIDS drugs. For example, the researchers found a small pocket that, if plugged by a drug, might shut down reverse transcriptase, and thus halt viral replication. This pocket is a possible target for future anti-AIDS drugs.

The work will also improve current understanding of how similar proteins in other viruses work, and how these proteins might contribute to other types of drug-resistant viruses. The new knowledge is of major importance: More than 33 million people worldwide are infected with HIV/AIDS. Almost 14 million people have died from the disease. [secondary B treatment]

Huang H, Chopra R, Verdine GL, and Harrison SC: Structure of a Covalently Trapped Catalytic Complex of HIV-1 Reverse Transcriptase: Implications for Drug Resistance. Science 282: 1669-75, 1998.

Balter M: Outsmarting HIV Drug Resistance. Science 282: 1623-5, 1998. (News section)

Borman S: Catalytic complex of HIV enzyme analyzed. Chemical and Engineering News, p. 7-8, November 30, 1998. (News section)

### **Gene Involved in Anesthesia Response Identified**

*Background:* Despite 100 years of trying, scientists have been unable to pin down the molecular targets of an important class of medicines used to anesthetize patients. The medicines, called volatile general anesthetics, are inhaled and spread quickly throughout a patient's bloodstream. The drugs' effects include relieving pain and keeping the patient still during surgery. To better understand how this vital yet mysterious group of medicines works, scientists have been hunting for the target molecules in the body that help the drugs induce anesthesia.

*Advance:* For the first time, basic researchers have found a gene encoding a protein that is required for the effects of the volatile general anesthetics isoflurane and halothane. The gene, called syntaxin, directs the production of a protein that helps communicate electrical impulses in nerve cells, and was discovered in a worm called *C. elegans* that is frequently used in laboratory research. An exquisite genetic tool, this tiny worm harbors a nervous system very similar to that of humans, but that is much smaller and easier to study. The researchers are continuing to use this model system to track down other anesthesia-related genes.

*Implications:* The human version of syntaxin is very similar to that of the worm. Moreover, scientists believe that the molecular interactions in which syntaxin participates in the nervous system of worms closely mimic those taking place in humans. Syntaxin is the first gene to be unearthed that is needed to carry out the effects of general anesthetics, and thus may point researchers to molecular targets for anesthesia. This discovery should further scientists' understanding of how anesthesia occurs, as well as perhaps help researchers develop improved anesthetic medicines. [secondary B treatment]

van Swinderen B, Saifee O, Shebestor L, Roberson R, Nonet ML, and Crowder CM: A neomorphic syntaxin mutation blocks volatile-anesthetic action in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 96:2479-84, 1999.

### **Fruit Fly Research Links Cocaine Sensitization With Biological Clocks**

*Background:* Cocaine is one of the most powerfully addictive street drugs. It can be taken by a variety of routes--abusers swallow, sniff, inject, or inhale cocaine to produce an intense, pleasurable "high." Cocaine produces its effects by hijacking key elements of the brain's communications system. By blocking the normal recycling of an important neurotransmitter in the brain called dopamine, cocaine leads to the overstimulation of certain brain regions, including those that control pleasurable behaviors. The basic features of human brain communication circuits are very similar to those in animal model systems, making the use of such systems an attractive and often inexpensive means to unravel some of the mysteries of the human brain. In recent years, a fruitful avenue for studying the molecular factors driving neurotransmission has been the study of insect nervous systems, using cocaine as a tool. To this end, last year basic researchers discovered that normal fruit flies become "sensitized" to cocaine--this behavior occurs not only in fruit flies but also in vertebrates and has been linked to drug addiction in humans.

*Advance:* A surprising new fruit fly study by the same group of researchers shows that cocaine sensitization is linked to genes that control the body's biological clock. The researchers now report that fruit flies missing several genes that play a critical role in the insects' internal biological clock do not become sensitized to cocaine. Scientists think that cocaine sensitization may underlie the increased drug craving, as well as the paranoia and psychosis, that occur in long-time cocaine addicts.

*Implications:* Besides potentially enabling the development of drugs to treat cocaine addiction, the new research holds out the prospect that biological clock genes might have other, as yet undiscovered, roles in the body. The work also links two research areas that previously had no apparent connection, and as such opens up the field of drug studies to thinking about how a totally unexpected set of genes functions in response to drugs. [secondary B treatment]

Andretic R, Chaney S, and Hirsh J: Circadian genes are required for cocaine sensitization in *Drosophila*. Science 285: 1066-8, 1999.

## Potassium Channel Research May Shed Light on Heart Ailment

*Background:* In order to function, many of our cells--including those in our heart, brain, and digestive tract--need to control the flow of potassium across their membranes. This flow is regulated by channel proteins--literally proteins that form channels through cellular membranes. Potassium ions flow freely into or out of cells when the channels are open. The channels open and close in response to biochemical stimuli.

*Advance:* Last year, basic researchers got a closer look at these potassium channels. Using a technique called X-ray crystallography, the scientists were able to determine the three-dimensional structure of a potassium channel molecule. This year, the same researchers examined the chemical properties of the molecule.

Based on the simple principle that ions, such as potassium, are normally repelled from the oily membrane of cells, the researchers asked: How does this channel protein allow potassium ions to cross the membrane? They knew from last year's research that, to accommodate the water-loving potassium ions that pass through it, the channel protein actually maintains a watery environment at its core. This year they learned that the electrical properties of the protein's center are perfectly tuned for the potassium ion.

Recently, the researchers also determined the structure of a separate section of the potassium channel, called the beta subunit. This part of the channel is not inserted through the membrane, but lies inside the cell, in contact with the rest of the channel protein. It is thought to help control the chemistry of the cell, which is linked with the opening and closing of the channel.

Finally, the scientists determined part of the structure of a specialized potassium channel that helps regulate heart rhythm. Defective versions of this channel underlie one form of long QT syndrome, a genetic condition that causes irregular heartbeat and sudden death.

*Implications:* Potassium channels are critical for many bodily functions, including heartbeat, nerve signaling, digestion, and insulin release. A better understanding of potassium channels may help scientists develop drugs to treat diseases ranging from heart ailments to diabetes. [secondary B treatment]

Roux B, and MacKinnon R: The cavity and pore helices in the KscA K<sup>+</sup> channel: Electrostatic stabilization of monovalent cations. Science 285: 100-2, 1999.

Gulbis JM, Mann S, and MacKinnon R: Structure of a voltage-dependent K<sup>+</sup> channel  $\beta$  subunit. Cell 97: 943-52, 1999.

Cabral JHM, Lee A, Cohen SL, Chait BT, Li M, and MacKinnon R: Crystal structure and functional analysis of the HERG potassium channel N terminus: A eukaryotic PAS domain. Cell 95: 649-55, 1998.

### **Inhibiting New Blood Vessel Growth Reduces Atherosclerotic Plaque in Mice**

*Background:* In atherosclerosis, coronary arteries and other blood vessels become obstructed by deposits of plaque (masses containing cholesterol and other lipid substances). Accordingly, much research has focused on developing ways to promote angiogenesis (growth of new blood vessels) in order to circumvent coronary arteries obstructed by the plaque deposits. Recent studies, however, suggest that the development of atherosclerotic plaques may be associated with the formation of new blood vessels, and researchers are now also turning their attention to inhibiting blood vessel growth.

*Advance:* New research indicates that angiogenesis may actually contribute to the progression of atherosclerosis. Investigators fed mice that already had plaque in their arteries a diet designed to mimic the cholesterol content of the typical diet eaten in the United States. Prolonged treatment with endostatin, a substance previously shown to inhibit the development of new blood vessels, reduced aortic plaque growth in this mouse model of atherosclerosis. Treated mice experienced, on average, an 85 percent reduction in the volume of plaque growth, compared with untreated mice. Mice treated with another angiogenesis inhibitor had an average 70 percent reduction in the volume of plaque growth.

*Implications:* These findings strongly suggest that the formation of new blood vessels is an important prerequisite for atherosclerotic plaque growth. With this knowledge, scientists may be able to develop new treatments to slow the development of atherosclerosis, thereby delaying the progression of heart and vascular diseases, and perhaps reducing the incidence of heart attacks and strokes. [secondary B treatment]

Moulton K, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J: Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 99: 1726-1732, 1999.

### **Combination of Therapies May Reduce Risk of Coronary Heart Disease in Women**

*Background:* Each year, about 250,000 American women die of coronary heart disease. Typically, heart disease develops 10 years later in women than in men. The reason for this is tied to women's production of the hormone estrogen; when women go through menopause, their ovaries essentially stop making estrogen and their risk of heart disease rises sharply. Researchers believe that this phenomenon is due, in part, to the beneficial influence of estrogen on a woman's cholesterol profile.

*Advance:* A recent study suggests that the addition of estrogen replacement therapy to cholesterol-lowering drug treatment has an extra protective effect against heart disease for postmenopausal women with high cholesterol levels. Results in 28 postmenopausal women with high cholesterol showed that combining the two therapies improved their cholesterol profiles (by lowering the bad low-density lipoprotein cholesterol and elevating the good high-density lipoprotein cholesterol) to a greater degree than either therapy used alone. In addition, only therapies including estrogen improved the capacity of the blood vessel wall to break down blood clots and to resist inflammation, two processes important for impeding the progression of atherosclerosis.

*Implications:* This research may lead to a new approach for treating healthy, postmenopausal women who have elevated cholesterol levels; the optimal approach may include estrogen therapy even for women who are already on cholesterol-lowering therapy. By reducing the risk of developing atherosclerosis, this combination of therapies could reduce the risk of heart attacks and strokes, thereby resulting in improved quality of life and cost savings from prevention of hospitalizations and the need for surgery. [secondary B treatment and prevention]

Kwang KK, Cardillo C, Minh NB, Hathaway L, Csako G, Waclawiw MA, Panza JA, Cannon RO: Vascular effects of estrogen and cholesterol-lowering therapies in hypercholesterolemic postmenopausal women. Circulation 99:354-360 1999.

## **Diet High in Fatty Fish May Protect Against Sudden Cardiac Death**

*Background:* Ventricular fibrillation, a disturbance in the rhythm of the heart, can cause sudden cardiac death (SCD). Each year, more than 250,000 individuals in the United States die of SCD due to ventricular fibrillation, and 55 percent of those deaths occur unexpectedly in individuals with no history of heart disease.

*Advance:* Evidence now suggests that fish oil, consisting of omega-3 ( $\omega$ -3) polyunsaturated fatty acids, reduces the incidence of life-threatening arrhythmias that result in SCD by directly affecting cardiac electrical activity. One recent study demonstrated that the protective effect (measured by reduction in all-cause mortality) of a Mediterranean-like diet that was high in  $\omega$ -3 fatty acids in a group of patients who had previously experienced a heart attack continued for at least 4 years. Recent results from other studies performed with dogs show that introduction of emulsified  $\omega$ -3 fatty acids directly into a vein significantly reduced the incidence of ventricular arrhythmias associated with SCD following experimentally induced heart attacks. These results, combined with those of the dietary study, indicate that  $\omega$ -3 fatty acids directly affect cardiac electrical activity.

*Implications:* The overall incidence of SCD might be markedly reduced by a diet that includes fatty fish. Since there are currently no effective therapies to prevent SCD in individuals with no history of heart disease, any reduction in the incidence of SCD would be a tremendous step forward. Two ongoing clinical trials are presently testing the anti-arrhythmic effectiveness of a diet high in fish oil. [secondary B prevention]

Leaf A: Dietary prevention of coronary heart disease: The Lyon Diet Heart Study. Circulation 99: 733-735, 1999.

de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N: Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 99: 779-785, 1999.

Billman GE, Kang JX, Leaf A: prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. Circulation 99(18):2452-7, 1999.

### **New Understanding of Increased Risk of Blood Clots in Old Age**

*Background:* Blood coagulation potential gradually increases through adulthood, reaching an almost 2-fold increase by old age. Increases in factors that promote coagulation, such as factor IX, appear to coincide with the increased blood coagulation potential. However, little is known about the molecular mechanisms that are responsible for this normal aging phenomenon.

*Advance:* Researchers have now identified two essential age-regulatory elements, AE5' and AE3', that are responsible for normal age-regulation of the human factor IX gene. Using a transgenic mouse model, they showed that the AE5' element is responsible for age-stable expression of the gene and the AE3' controls its age-associated elevation in expression. Together these two elements explain the increases in factor IX expression levels observed with aging.

*Implications:* Thrombotic disorders (i.e., formation of blood clots within the vessels, which often leads to heart attack or stroke) are a major health concern in the growing elderly population. An age-associated increase in coagulation potential may contribute to the increased risk of these disorders that accompanies aging. Factor IX is one of the coagulation factors that appears in increasing levels in plasma as people age. This genetic study has identified the regulatory elements responsible for the age-associated elevation in expression of the gene for factor IX, which leads to increasing levels of plasma factor IX with age. This work provides a new avenue to understanding age-related physical disorders and identifies potential target sites for new therapeutics for thrombotic disorders. [secondary B treatment]

Kurachi S, et al: Genetic Mechanisms of age regulation of human blood coagulation factor IX. Science 285:739-743, 1999.

## **Insight into How Hepatitis C Virus Evades Host Defenses May Lead to New Therapies**

*Background:* Hepatitis C virus (HCV) is an emerging virus of great medical importance that frequently causes chronic infection and may cause cirrhosis and even death. Interferon (IFN) therapy has been the principal treatment for patients suffering from chronic HCV infection, but most U.S. strains of HCV are resistant to this drug, with only about 20 percent of patients clearing the infection. The reasons for this poor outcome have not been understood. IFN normally acts by causing the cell to synthesize several antiviral proteins including one called double-stranded RNA-activated protein kinase (PKR). PKR acts to inhibit protein synthesis and in so doing would normally inhibit the virus. Investigators already knew that some cases of HCV resistance to PKR involved specific genetic mutations in an HCV protein called NS5A. The mutation in NS5A causes it to bind to IFN and block production of PKR. The changes in NS5A seemed to occur after initiating IFN therapy. However, it was already known that some isolates of HCV had inherent resistance to IFN.

*Advance:* Investigators recently determined that HCV has a second molecular strategy to evade IFN. They studied a protein that appears on the surface of the virus, E2, and found that it had a 12 amino acid sequence which is identical to a key interaction site (the phosphorylation site) in the interferon-inducible protein PKR. The E2 sequence is highly conserved among HCV strains suggesting it is important to the virus. Molecular action studies demonstrated that the E2 region inhibits an important biological activity of PKR (kinase activity), thus blocking the beneficial antiviral effects of IFN. Additionally, there is a direct relationship between interferon's effectiveness and the similarity of the E2 and phosphorylation site sequences. Patients who have type 1 HCV are most resistant to the curative powers of interferon; the sequence of E2 in type 1 HCV is closest to the sequence of the phosphorylation site.

*Implications:* HCV appears to have evolved two mechanisms to avoid the antiviral effects of IFN, one that is innate in the virus strain, and one that is induced after IFN treatment. Types of innate molecular mimicry are used by a number of pathogens to trick the body and avoid the natural host defenses. Understanding the ways in which a virus evades a host's natural defenses should provide new insights into designing therapies that attack the virus at unique molecular pathways. Recognition of specific virus targets also should help in the design of targeted drugs with fewer side effects. [secondary B treatment]

Taylor DR, Shi ST, Romano PR, Barber GN & Lai MMC: Inhibition of interferon-inducible protein kinase PKR by HCV E2 protein. Science 285:107-110, 1999.

### **Molecular Basis for Malaria Resistance and Susceptibility in Pregnant Women Defined**

*Background:* More than 40 percent of the world's population is at risk for malaria. The annual incidence of disease is estimated by the World Health Organization to be 300-500 million cases with 1.5-2.7 million people dying annually. Women are at increased risk from malaria during pregnancy; this risk is greatest during the first pregnancy. The reason for this increased risk is not known. Given the incidence of malaria worldwide and the need to protect millions of pregnant women and infants in the tropics, the question is an important one.

*Advance:* Investigators previously found that *Plasmodium falciparum*, the malaria parasite, adheres to a molecule called chondroitin sulphate A (CSA) on the surface of specialized placental cells called syncytiotrophoblasts. Consequently, the parasite binds to the cells in the human placenta. Using sera from women in Kenya, Malawi, and Thailand, investigators now have shown that certain antibodies (anti-adhesion antibodies) specifically block the CSA-dependent adherence of parasites, limiting the accumulation of parasites in the placenta. This type of antibody appears in pregnant women from Africa and Asia who have been pregnant on previous occasions, but not in those who are pregnant for the first time. Further studies showed that the presence of anti-adhesion antibodies against CSA-binding malaria parasites is associated with decreased risk of placental infection and lower levels of parasites in the blood and that the anti-adhesion activity is independent of the parasite strain.

*Implications:* Malaria susceptibility during first pregnancy may be related to the lack of these specific anti-adhesion antibodies. Unlike antimalarial antibodies measured by other types of assays, anti-adhesion antibodies appear to be associated with prevention of infection and appear to be strain independent. Both of these properties suggest that an anti-adhesion vaccine may protect against maternal infection. Strain independence of these antibodies would make a single type of vaccine useful regardless of the specific parasite in a geographic region. Additional research is needed to identify the malaria molecule with which these antibodies react as a further step toward vaccine development. [secondary B prevention]

Fried M, Nosten F, Brockman A, Brabin BJ & Duffy, PE: Maternal antibodies block malaria. Nature 395:851-852, 1998.

### **Molecular Studies of Hepatitis C Virus Identify Sequences that May Be Targets for Preventive Agents**

*Background:* More than 170 million people worldwide are chronically infected with hepatitis C virus (HCV). In many countries, HCV is the most common cause of chronic liver disease and hepatocellular carcinoma. HCV-related liver failure frequently requires liver transplantation. Given the scope of this public health problem, there is a great need to better understand the virus and to develop vaccines and effective treatments. HCV research has been difficult because a number of research methods used to study viruses are not possible with HCV. HCV does not replicate sufficiently in cell culture to allow biochemical analysis and so the identification of virus protein functions has relied on indirect analysis using artificial systems and comparisons with related viruses that are more amenable to study. A further complication is that the only validated animal model is the chimpanzee -- an endangered species. This severely limits the possible number of *in vivo* studies (studies in animals) and restricts them to sequential inoculation of the same animal.

*Advance:* Despite the impediments to studying HCV functions, investigators were able to identify specific regions of the HCV genome that are necessary for the virus to cause disease. They did this by using a complicated series of molecular-biologic and genetic techniques in which specific alterations were made in the virus' genetic material. In this case, investigators caused mutations that delete various discrete subregions of the virus' genetic material -- subregions that are not templates for specific virus proteins. By analogy with viruses related to HCV, the subregions are important for the replication of the virus genetic material, for the synthesis of the virus proteins, and for the assembly of the virus particle. The only way to test to see if these subregions affected the ability of the virus to cause disease was to inoculate them sequentially into the same chimpanzee. Investigators started with the mutant molecule they thought least likely to cause disease, injected it, and monitored for signs of disease. When no disease was observed, they proceeded with the next mutant molecule. Using this process of elimination, they identified two regions, called the conserved region and the poly (U-UC) region, that are critical for infectivity in the animals. Another region, the variable region, could be altered without an apparent effect on infectivity.

*Implications:* The studies provide significant information about parts of the HCV genome that are required for producing proteins important to virus functions. This knowledge is useful in identifying virus sequences that may provide novel and specific targets for preventive agents for HCV. [secondary B prevention]

Yanagi M, St. Claire M, Emerson SU, Purcell RH & Bukh J: *In vivo* analysis of the 3' untranslated region of the hepatitis C virus after *in vitro* mutagenesis of an infectious cDNA clone. Proceedings of the National Academy of Sciences 96(5): 2291-2295, 1999.

## **Maternal HIV Blood Levels Are Strong Predictors for Risk of Perinatal Transmission**

*Background:* Several studies have identified risk factors for mother-to-child (perinatal) transmission of HIV-1, including low birth weight, illicit drug use, and lack of prenatal treatment with the anti-HIV drug zidovudine (AZT). However, little was known about the relative importance of these risk factors, particularly in pregnant women receiving AZT.

*Advance:* Two separate teams of researchers have provided compelling evidence that the amount of HIV in a pregnant woman's blood, known as the maternal HIV viral load, is the prime risk factor for transmitting the virus to her infant. Both research teams found that infants born to women with the highest viral loads—whether they had received AZT or not—had the highest rates of HIV infection, while infants born to women with undetectable levels of HIV in their blood avoided infection. Maternal viral load remained a strong predictor for perinatal transmission even after accounting for other known risk factors.

*Implications:* This knowledge of the relative contribution of maternal viral load to the risk of perinatal HIV transmission is important because this risk factor can be modified. Scientists suggest that treating HIV-infected pregnant women with antiviral therapies that reduce their HIV blood levels to below the limits of detection should reduce their risk of transmitting the virus to their infants. [secondary B prevention]

Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, Burchett SK, Kornegay J, Jackson B, Moya J, Hanson C, Zorrilla C & Lew JF: Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. The New England Journal of Medicine 341(6): 394-402, 1999.

Mofenson, LM, Lambert JS, Stiehm ER, Bethel J, Meyer III, WA, Whitehouse J, Moya Jr, J, Reichelderfer P, Harris, DR, Fowler MG, Mathieson BJ & Nemo, GJ: Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. The New England Journal of Medicine 341(6): 385-393, 1999.

## **Latent Infection of CD4+ T Cells Provides a Mechanism for Lifelong Persistence of HIV-1**

*Background:* After protease inhibitors were added to multi-drug therapies for HIV infection, the United States AIDS death rate dropped for the first time since the beginning of the HIV/AIDS epidemic. The new combination therapies, known as highly active antiretroviral therapy (HAART), can suppress the replication of HIV-1 to the extent that the virus is undetectable in the blood, thus allowing many patients to live a longer, healthier life. The dramatic reductions in the level of HIV Aviral load@ raised hopes that HIV infection could be eradicated and infected patients could be Acured@ after several years of HAART. However, scientists remained concerned that HAART might not wipe out all traces of HIV and that latent reservoirs of cells infected with the virus could be activated if HAART were discontinued.

*Advance:* Several groups of researchers exploring this possibility found that certain immune cells can harbor a latent form of HIV-1 even in HAART patients who have had an undetectable viral load for 20 months or more. Collectively, these researchers contributed to an understanding of ongoing HIV-1 replication that has important implications for future research and treatment of HIV-infected patients. Now it is known that HIV infects active CD4+ T ("memory") cells in the immune system and that latent HIV infection is established if these cells survive and go back to a resting state. Because the biological function of memory cells is to respond to previously encountered antigens (substances that trigger an immune response), they have a long life span. Although these resting CD4+ T cells do not replicate while they are inactive, they form a long-term, latent reservoir of HIV that will replicate if these memory cells are reactivated. Scientists estimate that it will take more than 3 years for half of the infected cells CD4+ T cells in this latent reservoir to die. This slow rate of loss means that the eradication of a reservoir of only 100,000 HIV-infected cells could take more than 60 years in patients on current standard-of-care therapy. Because the time required for the elimination of the reservoir is so great, intervening problems such as cumulative drug toxicities or emerging drug resistance may make it impossible to eradicate HIV-1 infection with currently approved antiretroviral therapies.

*Implications:* This enhanced understanding of HIV replication and the limitations of HAART underscore the importance of finding other ways to eliminate the last vestiges of HIV in patients undergoing intensive antiretroviral therapy. Scientists will need to develop more effective and less toxic long-term medications to suppress HIV replication. Strategies are needed to accelerate the death of latently infected cells, to boost the immune system's response to HIV released from reservoirs of these cells, and to purge all residual virus from the body. Another important result of these studies is development of new technologies to verify eradication of HIV, should it ever occur. This ability will become increasingly important as new and improved drugs more effectively shrink the latent reservoir of HIV. [secondary B treatment]

Finzi D, Blankson J, Siliciano J D, Margolick JB, Chadwick K, Pierson T, Smith K, Lisziewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant, J & Siliciano, R F: Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nature Medicine 5(5): 512-517, 1999.

Furtado MR, Callaway DS, Phair JP, Kunstman KJ, Stanton JL, Macken CA, Perelson AS & Wolinsky SM: Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. The New England Journal of Medicine 340(21): 1614-1622, 1999.

*FY99 NIH GPRA Research Program Outcomes*

Zhang L, Ramratnam B, Tenner-Racz K, He Y, Vesanen M, Lewin S, Talal A, Racz P, Perelson, AS, Korber, BT, Markowitz, M & Ho, DD: Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. The New England Journal of Medicine 340(21): 1605-1613, 1999.

### **Identification of Salivary Anti-Thrombin from the Anopheles Mosquito May Lead to the Development of Novel Malaria Pharmaceuticals**

*Background:* More than 40 percent of the world's population is at risk for malaria. The malaria parasite is carried by the *Anopheles* mosquito. In the past, only the bite of these insects was considered important in the transmission of malaria to humans. However, more recent studies indicate that *Anopheles* mosquitoes actively contribute to the disease process. Investigators found that there are compounds in the saliva of blood feeding insects that affect the human body's normal processes for arresting bleeding (anti-hemostatic compounds) and the body's normal immune responses to foreign materials (anti-inflammatory compounds). While this interference with normal human functions allows the insect to feed efficiently, it also enhances the transmission of insect-borne pathogens such as malaria.

*Advance:* Using a variety of bioassays, purification methods, and sequencing techniques, investigators isolated a peptide called anophelin from the salivary glands of the *Anopheles albimanus* mosquito. They cloned and synthesized the enzyme and showed it to be a specific and novel inhibitor of thrombin, a substance with a key role in blood clotting.

*Implications:* Thrombin functions in the blood-clotting cascade. Thus, the presence of an anti-thrombin activity in insect saliva prevents clotting at the site of the insect bite. While this enhances the mosquito's ability to get its blood meal, anophelin has the potential to facilitate the transmission of parasites carried by the insect, by facilitating a pathway between the carrier and the person being bitten. The identification of biologically active molecules from mosquitoes has the potential to provide new prevention and treatment modalities. Vaccines could be designed to interact with anophelin, inhibiting or reducing its activity, and, thus, diminishing transmission. Another conceivable application of this insect molecule is in development of a new class of anti-coagulants. [secondary B treatment]

Valenzuela JG, Francischetti IMB & Ribeiro JMC: Purification, cloning, and synthesis of a novel salivary anti-thrombin from the mosquito *Anopheles albimanus*. Biochemistry 38(34): 11209-11215, 1999.

### **Knowledge about an Immune Cell Receptor Type Provides Clues about Immunoregulatory Processes that Affect Pregnancy**

*Background:* Natural Killer (NK) cells provide an important immune function in the body's defense against infection by viruses and other pathogens that replicate inside of cells. Unlike the other major types of white blood cells (called B and T cells), NK cells do not show specificity for a particular foreign molecule (antigen); they were given their name because of their ability to kill other cells without having prior exposure (stimulation) by a specific antigen. The killing of normal healthy cells is prevented by inhibitor molecules (receptors) on the surface of NK cells that recognize particular host proteins (self-antigens) called major histocompatibility class I molecules (HLA-class 1). The HLA-class 1 molecules are perhaps best known as the proteins that determine if an organ transplant will be accepted or rejected. In general, the HLA antigens of the donor and recipient must match; otherwise, the recipient's immune system will perceive the transplanted organ as foreign and reject it. During pregnancy, the developing fetus has some properties similar to that of a transplanted organ. That is, the fetus inherits paternal, as well as maternal, HLA antigens. The paternal antigens could be seen by the maternal immune system as foreign, if they do not match the maternal ones. The mechanism by which the woman's immune system refrains from reacting to the fetus is unknown.

*Advance:* Investigators studied the receptors on NK cells that inhibit their natural cell killing (cytotoxic) ability -- killer cell immunoglobulin (Ig)-like receptors (KIRs). They identified a particular KIR, KIR2D4L, that is structurally different from other KIRs. KIR2D4L is unique in that it is produced on all NK cells, even though it binds only to cells that present one particular HLA type, HLA-G. Because HLA-G is produced only on specific fetal cells (trophoblasts) that interact with maternal cells at the maternal-fetal interface, KIR2D4L may provide regulatory signals to the maternal NK cells. Further studies are needed to determine the mechanism(s) of interaction between the cells.

*Implications:* These basic studies have led to an important observation that sheds light on how the immune system controls critical interactions between natural killer cells and trophoblast cells during early pregnancy -- interactions which may ensure the formation of a successful implantation site for the developing embryo. Thus, the advance could illuminate early pregnancy loss. In addition to elucidating a very specific function, fetal immune privilege, as a clue to the mechanisms for achieving tolerance to specific antigens, this knowledge provides insights on global aspects of immune regulation.

Rajagopalan S & Long EO: A Human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. Journal of Experimental Medicine 189(7); 1093-1099, 1999.

## **Natural Model of Cutaneous Leishmaniasis Provides Insights into Vector's Role in Pathogenesis and Potential for Novel Prevention Strategies**

*Background:* Worldwide, the numerous *Leishmania* species cause a spectrum of diseases in humans with pathologies ranging from asymptomatic to lethal. This protozoan parasite is transmitted by sand flies (*Phlebotomus papatasi*). Cutaneous leishmaniasis causes a range of skin lesions, which can become secondarily infected with bacteria. In the past, the insects that transmit the disease to humans were considered to be flying syringes.<sup>@</sup> More recent studies indicate that they contribute actively to the disease process. An initial clue for these studies was the observation that small amounts of sand fly saliva could enhance infection when co-inoculated with *Leishmania* organisms.

*Advance:* Investigators have established and extensively studied a murine (mouse) model of cutaneous leishmaniasis infection. The model involves transmission of *Leishmania major*, which causes cutaneous leishmaniasis, through sand fly bites on mouse ears. To explore the effect of sand fly saliva on the immune response to *L. major*, investigators exposed healthy mice to saliva from uninfected sand flies via intradermal inoculation of the mouse ear with extracts of the sand fly salivary glands. Subsequently, mice were exposed to saliva containing *Leishmania* organisms. The results indicated that components in sand fly saliva exacerbate lesion development at the site of the bite. The molecules in the sand fly saliva contribute to lesion formation by inducing mouse cells to produce immune modulators, called cytokines (in this case interleukins-4 and -5). Mice that were inoculated with uninfected sand fly saliva previous to exposure to the *L. major* made antibodies to the insect saliva components. Consequently, when those mice were subsequently exposed to saliva containing *Leishmania* organisms, the saliva did not induce the mice to generate the cytokines that exacerbate lesion formation. Additional studies with mice having a variety of immune defects indicated that the insect saliva affected multiple components of immune pathways.

*Implications:* This model has important implications for prevention strategies. It demonstrates that the saliva of the insect vector influences the immune response that a person has and shows that the presence of anti-saliva antibodies in the host can diminish disease. Thus, *Leishmania* vaccines may need to incorporate not only proteins from the *Leishmania*, but also from the transmitting sand fly saliva. [secondary B prevention]

Belkaid Y, Kamhawi S, Modi G, Valenzuela J, Noben-Trauth N, Rowton E, Ribeiro J & Sacks DL: Development of a natural model of cutaneous leishmaniasis: Powerful effects of vector saliva and saliva preexposure on the long-term outcome of *leishmania major* infection in the mouse ear dermis. Journal of Experimental Medicine (188) 10: 1941-1953, 1998.

### **Sequencing of Organism involved in Sexually Transmitted Diseases and Blindness Provides Clues for Vaccine and Drug Development**

*Background:* The bacterium *Chlamydia trachomatis* causes several diseases of medical importance. Ocular infection leads to trachoma, a leading cause of preventable blindness. Chlamydia genital tract infections are among the most common of infectious diseases reported to the Centers for Disease Control and Prevention (CDC). They can result in pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain, epididymitis, and infant pneumonia. *C. trachomatis* genital tract infections may also increase the risk for HIV infection.

*Advance:* NIH-supported researchers have sequenced the genetic material of *C. trachomatis*. Computer comparison of these sequences with other more thoroughly studied bacteria, and use of algorithms to predict DNA sequences that code for proteins have provided new insights into chlamydia pathogenesis and vaccine and treatment candidates. Chlamydia are called obligate intracellular parasites because they live within host cells. Analysis of the genome revealed that although chlamydia lack many biosynthetic capabilities, they retain functions for utilizing nutrients obtained from host cells.

*Implications:* This research is an example of the use of molecular technologies to study an important human pathogen. A combination of biochemical and computer studies has identified unique essential proteins and pathways that can provide targets for drugs, antigens to include in vaccines, and molecules useful for diagnostics. [secondary B treatment, prevention, and diagnosing]

Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, Mitchell W, Olinger L, Tatusov RL, Zhao Q, Koonin EV & Davis RW: Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science 282: 754-759, 1998.

### **Preventing Unwanted T Cell Activation by Triggering CTLA-4, A Negative-Signaling Molecule**

*Background:* T cells are white blood cells directly involved in the body's immune defense system. T cells recognize transplanted organs as "foreign" and mediate transplant rejection. In addition, inappropriate activation of T cells can result in autoimmune diseases, such as systemic lupus erythematosus (SLE) and type 1 diabetes. A negative signaling molecule on the surface of T cells, called CTLA-4, normally prevents unwanted and self-destructive T cell activity. Elucidation of this and other negative signaling pathways will lead to the development of new approaches to induce immune tolerance (the selective lack of an immune response to specific antigens) by blocking signals for T cell activation.

*Advance:* Researchers proposed to prevent inappropriate immune responses by using the negative signaling of CTLA-4 molecules on T cells to stop self-destructive autoimmune responses and to induce immune tolerance to organ transplants. T cell activation was known to result from changes that occur in the molecule used by T cells to detect specific antigens, the T cell antigen receptor (TCR). In the presence of antigen, phosphate molecules are added to certain amino acid residues on the intracellular portion of the TCR, enabling the TCR molecule to function in T cell activation. Scientists discovered that CTLA-4 and TCR molecules interact on the membranes of T cells and that their association activates a particular enzyme that removes phosphate molecules from the activated TCR. Thus, they learned the mechanism by which CTLA-4 reduces T cell activity and turns off immune responses.

*Implications:* Understanding the molecular pathways of T cell activation will contribute to the development of new and more effective approaches to induce immune tolerance, thereby improving the success rate of organ transplants and the quality of life for transplant recipients. Additionally, knowledge of how the immune system is regulated can further the development of treatments for autoimmune diseases and contribute to the development of therapies for asthma and allergic diseases. These therapies could be new molecules to modulate the immune system or genetic therapies that would suppress or strengthen the immune response. [secondary B prevention]

Kyung-Mi L, Chuang E, Griffin M, Khattri R, Hong DK, Zhang W, Straus D, Samelson LE, Thompson CB & Bluestone JA: Molecular basis of T cell inactivation by CTLA-4. Science 282: 2263-2266, 1998.

### **The Adult Thymus Is Capable of Rebuilding the Immune System after Treatment of HIV Infection**

*Background:* The thymus gland is a major site for the production and generation of T cells, which play a critical role in the immune system's defenses. Scientists have known that thymic function declines with age and that these age-related declines may affect the ability of the thymus to reconstitute T cells that are lost during HIV infection.

*Advance:* In order to determine the level of thymic function, researchers developed an assay based upon previously defined patterns of T-cell receptor gene rearrangement. Before T cells are released from the thymus, they generate a genetic byproduct known as T-cell receptor rearrangement excision circles, or TRECs. Because TRECS are not reproduced during later cycles of cell division, the presence of these DNA fragments can identify T cells that recently left the thymus and thus could serve as a marker (indicator) of thymic output and function. Researchers determining the relationship between TREC quantity and age found that even persons as old as 73 years had detectable quantities of TRECs in their blood. Thus, T cells were still being produced by the thymus in older persons. This finding indicates that although thymic production of new T cells declines with age, it remains substantial into late adulthood rather than being limited to infancy and early childhood, as had been previously believed. Scientists also found that although adult patients infected with HIV had suppressed thymic function, most showed a progressive and sustained increase in new T cells following infection suppression with highly active antiretroviral therapy (HAART).

*Implications:* Evidence that the thymus is active in adults and that HIV-suppressed thymic function can be improved by HAART offers significant hope that an HIV-ravaged immune system may be able to rebuild itself after intensive antiretroviral therapy. Therapies that directly improve thymic function may also increase the rate of immune reconstitution after HAART. In addition, methods for tracking newly produced T cells are valuable tools for monitoring immune reconstitution in HIV-infected people. Finally, these findings are especially relevant for studies involving HIV-infected children. Because the thymus is much more active in children, monitoring thymic output in this population will provide valuable information on immune reconstitution after antiretroviral therapy. [secondary B treatment]

Jamieson BD, Douek DC, Killian S, Hultin LE, Scripture-Adams DD, Giorgi JV, Marelli D, Koup RA & Zack JA: Generation of functional thymocytes in the human adult. Immunity 10: 569-575, 1999.

Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, Polis MA, Haase AT, Feinberg MB, Sullivan JL, Jamieson BD, Zack JA, Picker LJ & Koup RA: Changes in thymic function with age and during the treatment of HIV infection. Nature 396: 690-695, 1998.

## **Insulin Resistance in Tissues Underlies Type 2 Diabetes**

*Background:* Type 2 diabetes affects more than 16 million Americans and is the predominant form of diabetes in the U.S. It is characterized by a resistance to the action of insulin--the hormone necessary for converting sugar, starches and other foods into energy-- in many different tissues of the body. This is coupled with the inability of the pancreas Beta cell to deliver insulin in a regulated pattern and quantity to control the breakdown of the sugar glucose. The underlying mechanism causing insulin resistance is poorly understood. In order to gain insight into how type 2 diabetes develops and progresses, it is critical to understand the signaling pathways used by the cell protein that binds insulin (the insulin receptor) and how abnormalities in this system lead to insulin resistance.

*Advance:* Investigators have applied a technique that uses specially developed genes to act as molecular scissors that can recognize, target and cut out segments of genetic material. In this case, the method was used to delete the insulin receptor in the pancreatic beta cell to address the question of whether disruption of insulin signaling in the beta cell could contribute to an alteration in its function. Mice showed a selective loss of insulin secretion in response to glucose and progressively impaired glucose tolerance. These findings provide direct evidence of a functional role for the insulin receptor in the maintenance and balance of glucose levels and suggest that defects in insulin signaling in the beta cell may contribute to the observed alterations in insulin secretion in type 2 diabetes.

*Implications:* These findings suggest a unifying hypothesis for type 2 diabetes in which insulin resistance in all insulin-responsive cells, including the beta cells, could result in the classical manifestation of disease in type 2 diabetes. In humans, combinations of environmental and genetic alterations in the insulin receptor and in the insulin signaling pathway could provide a mechanism for the development of type 2 diabetes. An understanding these events has implications for new approaches to both treat and prevent this disease. [secondary B prevention and treatment]

Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, and Kahn CR. , Tissue-Specific Knockout of the Insulin Receptor in Pancreatic  $\beta$  Cells Creates an Insulin Secretory Defect Similar to that in Type 2 Diabetes. Cell 1999; 96:329-339.

### **Diabetic Heart Disease Still a Disproportionate Problem**

*Background:* About sixteen million people have diabetes. Of these, one-third do not know they have it. Each year, about 800,000 people in the United States are diagnosed with diabetes, and the prevalence of diabetes has increased six-fold in the past thirty years. Heart disease is two to four times more common in adults with diabetes than in their nondiabetic counterpart, accounting for about fifty percent of deaths of people with diabetes. With the increasing prevalence of diabetes, it is expected that diabetes will become an increasingly important reason for heart disease mortality in this century.

*Advance:* Overall, deaths from heart disease have fallen dramatically in the United States, due to the improvement in heart disease risk factors and better medical care. Researchers examined whether these changes occurred to the same extent for diabetic patients through an analysis of mortality data from two national cohorts derived from the 1971-1975 First National Health and Nutrition Examination Survey (NHANES I) and the 1982-1984 NHANES I Epidemiologic Follow up Survey (NHEFS). They found that deaths from heart disease declined thirty-six percent in nondiabetic men during 1971-1993, but fell only thirteen percent in diabetic men. Heart disease actually rose by twenty-three percent in diabetic women, despite a twenty-seven percent drop in deaths from heart disease in nondiabetic women. The study also found that deaths from all causes in diabetic adults have not declined to the extent that they have for people who do not have diabetes.

*Implications:* These are staggering results, showing that a general decline in cardiovascular risk factors in the population, along with better treatment methods for heart disease, was not sufficient to deal with the diabetic disease process. Research on the reasons for these discrepancies is essential, especially for women, in order to find effective methods for the prevention of diabetes and its complications and to avoid the excess deaths by heart disease in diabetes. [secondary B prevention]

Gu K, Cowie CC, Harris MI, Diabetes and the decline in heart disease mortality in US adults.. Journal of the American Medical Association. 1999; 281:1291-97.

### **Increased Insulin Sensitivity and Obesity Resistance in Mice**

*Background:* Obesity is a major cause of morbidity and mortality in the United States. It is also a major risk factor for the development of type 2 diabetes, the predominant form of diabetes in the United States. Because type 2 diabetes is characterized by the body's inability to respond to insulin, the hormone necessary for converting glucose into energy, diabetics must follow a strict diet and exercise routinely in order to keep their blood glucose levels from rising out of control. Maintenance of blood glucose levels is also important to ward off development of the severe complications of diabetes--kidney disease, eye disease, heart disease, and nerve disease. Unfortunately, even such strict regimens do not work for everyone with type 2 diabetes and these individuals then require drugs to control their blood sugar levels. Five classes of oral medications, each of which works through a different mechanism of action, are currently available to improve blood glucose control in patients with type 2 diabetes. More effective, safe and cost-efficient drugs to treat type 2 diabetes are desperately needed.

*Advance:* NIH-supported researchers have identified a major new target for the possible development of such a drug. Investigators created a mouse model lacking an enzyme (protein tyrosine phosphatase-1B) that plays a role in the insulin signaling pathway. Ultimately, it tells a cell to take up glucose and store it, lowering blood glucose levels. Researchers found that removal of this enzyme effectively turned off the insulin signaling pathway, in the mouse model. Test mice were much more sensitive to insulin's blood sugar lowering effects. These findings raise the possibility of treating type 2 diabetes with drugs that block the activity of this enzyme. Interestingly, these mice also seemed to be able to consume a high fat diet without gaining much weight. This suggests that drugs that block the enzyme may also be useful for treating obesity. Equally important, the mice without the enzyme appear healthy.

*Implications:* Type 2 diabetes, for which obesity is a major risk factor, is characterized by the body's inability to respond to insulin. In almost all cases where an individual is obese and diabetic, successful treatment of obesity will also improve diabetes. Mice lacking an important enzyme are more sensitive to the hormone insulin, appear healthy, and seemed to be protected from obesity, making this enzyme a very exciting target for the treatment type 2 diabetes and obesity. Unknown is whether overactivity of the enzyme has a role in obesity in normal animals- or in people. This may not matter though, for the development of an anti-obesity drug. The prospect of a new, safe and effective drug to treat type 2 diabetes--along with the complicating condition of obesity--has the potential to advance the future practice of medicine. [secondary B treatment and prevention]

Elchebly M et al., Increased Insulin Sensitivity and Obesity Resistance in Mice Lacking the Protein Tyrosine Phosphatase-1B. Science 1999; 283: 1544-8.

Ferber D, New Clues Found to Diabetes and Obesity. Science 1999; 283: 1423, 1425.

### **Paracellin-1, a Renal Protein, is Required for Magnesium Homeostasis**

*Background:* The composition of fluids on opposite sides of membranes lining organ surfaces and spaces (epithelial membranes) is often different, constituting a transcellular barrier. The composition of fluids is maintained by barriers to passage of fluid, minerals (electrolytes), nutrients, toxins and pathogens, between cells and the extracellular fluid. In addition, contacts between membranes of cells provide a structural permeability barrier. The reabsorption and preservation of magnesium, which is a critical cofactor in many biologic activities that must be retained in the body and restricted in its excretion, is highly regulated and occurs by flux outside and between cells, in the paracellular space. Because the mechanisms underlying magnesium regulation and balance remain obscure, researchers examined the magnesium paracellular flux. These studies should not only shed light on magnesium regulation and balance, but also on the paracellular regulatory process itself.

*Advance:* In the kidney, magnesium reabsorption occurs in the structure known as the thick ascending limb of Henle (abbreviated TAL). This process is driven by favorable directional forces (an electrical and chemical gradient of forces). Recent gene cloning studies have identified a human gene coding for the protein Aparacellin-1.<sup>@</sup> Paracellin-1 was found to be located in the specific membrane contacts between cells, called tight junctions, of the TAL, suggesting that it may be a constituent of the pore-like structure that permits the paracellular flux of magnesium in the TAL, and that it serves as an essential component for selective paracellular regulation of magnesium.

*Implications:* This study contributes to the understanding of magnesium regulation and balance, and also of the paracellular regulatory process itself. The mechanism is important for its own sake, as well as for the knowledge of the processes of the kidney it offers. The results raise the possibility that other members of the tight junction protein family, called the Acludin<sup>@</sup> family, also mediate specific paracellular regulation and determine the permeabilities of different cell membranes. Finally, this research may benefit patients with the rare disease called Arenal hypomagnesemia,<sup>@</sup> which features profound renal wasting of magnesium and calcium leading to renal calcification and renal failure. [secondary B prevention and treatment]

Simon DB et al., Paracellin-1, a renal tight junction protein required for paracellular Mg<sup>2+</sup> reabsorption. Science 1999; 285: 103-106.

## **Types of Pituitary Gland Cells: How Are They Determined?**

*Background:* Six distinct cell types of the mature pituitary gland serve critical functions of metabolic balance in the body by regulating key hormonal (endocrine) organ targets in response to signals from the brain and from distal tissues. All of these cell types arise from a common precursor cell in a specific space and time sequence. The mechanisms by which signaling molecules lead to emergence of specific cell types is a central question for the understanding of organ development in man and other mammals. It is known that there is a distribution, or gradient, of levels of transcription factors, proteins that regulate which genes become active (gene expression) and control cell growth and function, across the lower pituitary and the upper pituitary. The hypothesis for the present study was that each gradient forms a transient pattern in space and time that determines formation of a specific cell type.

*Advance:* This research demonstrates that the differentiation of four pituitary cell types is mediated by the action of two transcription factors, called GATA2 and Pit1. There are distinct interactions between these two factors, both inhibitory and synergistic, which play a critical role in the events that determine the cell type formed, the exact time of formation and its position within the mature pituitary gland. These factors serve as the Amolecular memory<sup>®</sup> of these signaling events. This study also revealed for the first time that Pit1 acts in both a DNA binding-dependent and -independent manner; directly regulating the expression of GATA2 and inhibiting protein-protein interactions, which in turn control the function of other transcription factors.

*Implications:* Genetic and hormonal dysfunction in the pituitary gland is responsible for a wide range of human diseases. The regulation of the endocrine system depends on the development of special cell types within designated areas of the pituitary gland. Understanding these mechanisms is critical to development of effective interventions in genetic and endocrine disease. The study of pituitary development also serves as a model for the development of other organs and organ systems. The actions of Pit1, both DNA-dependent and DNA-independent, may prove to be a basis for or model of the actions of other highly expressed transcriptional regulatory factors in the development of human and other mammalian organs. [secondary B treatment and prevention]

Dasen JS et al., Reciprocal Interactions of Pit1 and GATA2 Mediate Signaling Gradient-Induced Determination of Pituitary Cell Types. Cell 1999; 97:587-98.

## **New Mechanisms of Resistance to Urinary Tract Infection are Identified**

*Background:* Urinary tract infections are among the most common infectious diseases acquired by humans and cause substantial morbidity. The main infectious culprit is the intestinal bacterium *Escherichia coli* (*E. coli*). The interior surface of the bladder, the lumen, is lined by a layer of superficial cells called umbrella cells<sup>®</sup>. Deposited on their upper, or apical, surfaces are crystalline-like proteins called uroplakins. Certain pathogenic strains of *E. coli* are known to have surface adhesive structure called type 1 pili. Recent evidence indicates that two of the uroplakins (UPLa and UPLb) can specifically bind *E. coli* with these pili on their surface.

*Advance:* New evidence indicates that one restraint on invasion of the bladder by piliated *E. Coli* is the induction of death of local bladder lining cells (apoptosis) and shedding of cells lining the bladder. A category of enzymes called caspases are important in induction of the programmed cell death, and interfering with caspases can prevent activation of cell death pathways. These studies looked at the structural basis and consequences of the interactions between type 1-piliated *E. coli* and the uroplakin-coated host bladder cells in mice. The bladders of the animals were infected directly with *E. coli* strain grown under conditions that specifically induces the expression of type 1 pili. It was found that treatment of mice with an chemical that blocked the action of caspase inhibited shedding of bladder lining cells in response to infection: At 6 hours after infection, the bladder cells remained mostly intact. However, massive exfoliation of the lining cells eventually occurred in the mice by 24 hours after infection. In addition, at 12 hours after infection, control mouse bladders had an average of 85% fewer bacteria compared with treated mice. These results support a role for shedding of the bladder lining in clearing the bacteria, and implicate a caspase-mediated cell death mechanism in the shedding of cells as a protective mechanism in response to bladder infections.

*Implication:* This study confirms that pathogenic *E. coli* induce programmed cell death and exfoliation of the cells lining the bladder, but that they can resist this innate host defense by invading into deeper tissue. This may account for the high amount of disease persistence or recurrence, despite antibiotic treatment, in many patients with urinary tract infections. Recurrent infections are a common problem that frequently affect women who have otherwise normal urologic health. The ability of these pathogens to invade into deeper tissue suggests that recurrences in some cases are manifestations of a lingering chronic infection and not a new infection. Some seven million people in the U.S. are affected annually. [secondary B prevention and treatment]

Mulvey MA et al., Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. Science 1998; 282: 1494-1497.

## Understanding the Regulation of Gene Expression

*Background:* Steroid hormones are small signaling molecules, or ligands, that control many aspects of human development and normal human function, including cell growth and metabolism. These ligands also play a role in many disease processes and their synthetic counterparts are used widely as therapeutic agents. They function by activating specific proteins called Nuclear receptor proteins found inside cells. The activated receptors then bind to a specific target part of a gene. After binding to target genes, the receptor requires the assistance of proteins called transcriptional co-activators in order to switch the gene on. The product of the activated gene then has the potential to affect cellular processes in a specific way--termed the biologic response. The nuclear receptor family of proteins also includes a number of additional proteins called orphan receptors because they do not have known ligands. The roles of these proteins are less well characterized, but it is believed that they also play important roles in diverse areas.

*Advance:* This year, NIH researchers reported a number of advances in nuclear receptor function. Researchers have isolated and characterized a novel transcriptional co-activator termed steroid receptor activator or SRA. SRA is different from other co-activators in that it does not exhibit characteristics of a protein, but rather characteristics of a ribonucleic acid (RNA), a chemical found in the nucleus and cytoplasm of cells that plays an important role in protein synthesis and other chemical activities of the cell. This RNA is specifically expressed in steroid target tissues and functions as a component of a larger multiprotein complex to selectively enhance gene expression by steroid receptors. Other investigators have identified a number of ligand-dependent proteins called Vitamin D Receptor-Interacting Proteins, or DRIPs, which interact with the receptor for vitamin D, another steroid hormone. Together, these proteins make up a new cofactor complex. The DRIPs are almost indistinguishable from components of another cofactor complex called ARC (for Activator-Recruited Cofactor). Several of the DRIP/ARC subunits also function as part of other cofactors, suggesting that unique classes of activators may share common sets of cofactors. Therefore, the role of nuclear receptor ligands may, in part, be to recruit a specific cofactor complex to the receptor, and in doing so, enhance expression of specific target genes. Lastly, researchers have found that the orphan nuclear receptor farnesoid X, or FXR, acts as a nuclear receptor for bile acids, natural products essential for the absorption of dietary lipids and for removal of toxic substances from the body. The identification of bile acids as natural FXR ligands suggests that these compounds may have important and unexpected functions in the regulation of gene expression, as well as mammalian lipid metabolism.

*Implications:* Taken together, these studies add to our understanding of the complexities of gene expression and the basic cellular functions underlying development. The last study provides direct evidence for a nuclear bile acid signaling pathway that may regulate cholesterol balance. Further, identification of specific co-activators and cofactors suggests novel therapeutic approaches in clinical settings. [secondary B treatment and prevention]

Lanz RB et al., A Steroid Receptor Coactivator, SRA, Functions as an RNA and is Present in an SRC-1 Complex. *Cell* 1999; 97:17-27.

*FY99 NIH GPRA Research Program Outcomes*

Rachez C et al., Ligand-dependent Transcription Activation By Nuclear Receptors Requires the DRIP Complex. Nature 1999; 398:824-28.

Parks DJ et al., Bile Acids: Natural Ligands for an Orphan Nuclear Receptor. Science 1999; 284:1365-68.

## Pathways In the Brain That Control Food Intake

*Background:* Obesity is a major cause of morbidity and mortality in the U.S. Overweight now affects more than one out of every three Americans, and its prevalence has increased thirty percent over the past decade alone. The prevalence of obesity is also increasing among children and adolescents. Obesity results from an imbalance between energy intake and energy expenditure. The coordination of energy intake and expenditure occurs through a variety of signals that originate from fat tissue, various regions of the brain, the endocrine (hormonal) system, and the gastrointestinal tract. Over the past few years, progress has begun to define these complex pathways. It is now known that leptin, the protein product of the obesity gene, is secreted by fat tissue. Leptin, together with other hormones such as melanocyte stimulating hormone (MSH) which acts on a region of the brain called the hypothalamus, suppress appetite. Other hormones, such as neuropeptide Y (NPY), melanin concentrating hormone (MCH), and a family of proteins called the orexins, act on other areas of the hypothalamus to stimulate appetite. The signaling pathways created by these hormones and their receptors are important in determining whether a person feels hungry or full, and how much the individual might eat in response to this feeling.

*Advance:* Melanocortin signaling has been recognized for its ability to darken skin color and fur color. It has also been identified as an essential part of normal body weight maintenance. Researchers have recently identified the mouse *Amahogany* gene, adding new understanding to the melanocortin system. Researchers have now shown that mahogany is widely expressed in human tissues, including pigment cells and the hypothalamus, and that it is a close relative to a human protein called attractin. Attractin is a circulating molecule produced by critical cells active in the immune response. This observation provides new insight into the regulation of energy metabolism and indicates a relationship between melanocortin-receptor signaling and immune function. Another study involving mahogany indicates that this gene influences energy balance. Researchers demonstrated that mahogany is expressed in the hypothalamus and that a mutation in the mahogany gene can prevent diet-induced obesity in mice.

As stated earlier, melanin-concentrating hormone (MCH) acts to stimulate appetite. Researchers have created a mouse model in which the MCH gene has been deleted. These mice have leanness and reduced body weight due to reduced feeding and an inappropriately increased metabolic rate, despite reduced levels of leptin.

*Implications:* This new information on hypothalamic pathways that can control food intake has led to fundamental advances in our understanding of body weight regulation. Second, the mahogany and MCH stories show that systems that control energy expenditure are also important in this process. Lastly, these studies have implications for the development of therapeutic agents that target these complex pathways to treat obesity. [secondary B prevention]

Gunn TM et al., The mouse mahogany locus encodes a transmembrane form of human attractin. Nature 1999;398:152-6.

Nagle DL et al., The Mahogany Protein is a Receptor Involved in Suppression of Obesity. Nature 1999;398:148-52

*FY99 NIH GPRA Research Program Outcomes*

Schwartz MW, Mahogany adds color to the evolving story of body weight regulation. Nat Med 1999;Apr;5(4):374-5.

Shimada M et al., *Mice lacking melanin-concentrating hormone are hypophagic and lean.* Nature 1998 Dec 17;396(6712):670-4.

## **Turning Brain into Blood Turning Bone Marrow into Liver Cells**

*Background:* Cells at an early stage of specialization (stem cells) are found in several adult organ systems, such as blood, intestine, and skin, where they are able to replace the more specialized or defined cells that may be lost to physiological turnover or injury. Stem cells have now been found in the central nervous system (CNS), where they can generate the three major cell types in the brain: neurons, astrocytes, and oligodendrocytes. An important unresolved question is whether stem cells in adult organs are limited to producing specialized cells of that organ, or whether they are capable of producing cell types needed in other organs as well. As evidence for the developmental potential of stem cells, these cells (in their early stages of differentiation) found in the bone marrow have been shown capable of developing into the cell lineages of blood vessels, muscle, fat, cartilage, and bone (mesenchymal tissues), but not, for example, into liver cells--at least until now.

*Advance:* Using cloning of adult CNS stem cells injected into irradiated mouse recipients (to eliminate blood-forming tissue) of a different strain than the stem cell donors, investigators found that after a period of 5 to 12 months, analytic tests were able to establish that the former neural stem cells had become blood-forming cells.

In the case of bone marrow stem cells, investigators used transplantation procedures to trace the origin of repopulating liver cells after liver injury in irradiated animals. They used several types of markers to identify liver cells of bone marrow origin. The limits and biological importance of the bone marrow-derived cells need to be assessed.

The results of these studies add to the growing body of evidence that stem cells in the adult organism have a remarkable ability to become adult cells different from those of the donor organ.

*Implication:* The demonstration of the developmental potential of stem cells in several systems indicates that stem cells in a specific location are capable of giving rise to blood-forming cells, and the converse. These reports suggest that truly primitive stem cells exist, and that these cells can respond to their environment in a tissue-specific manner. Further understanding of the potential of various stem cells for tissue regeneration and replacement is required for clinical application. [secondary B treatment]

Bjornson CRR et al., Turning Brain into Blood: A hematopoietic fate adopted by adult neural stem cells in vivo. Science 1999;283:534-537.

Petersen BE et al., Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168-1170.

## **Mechanisms of Female Sex Determination and Development**

*Background:* In the mammalian embryo, the two sexes are initially indistinguishable by form; specific hormones are required for sex-specific development. Correct sexual development depends on differentiation of cells in two simple ducts, the Müllerian and Wolffian ducts, respectively, into male or female structures. Male differentiation is triggered by the testis-determining factor, which is encoded by the Y chromosome, and which results in male differentiation, with development of the Wolffian duct, testosterone formation and regression of the Mullerian duct. Female differentiation, which is associated with degeneration of the Wolffian duct, has always been considered a default condition that results from the absence of the testis-determining factor and of testosterone. New evidence challenges this view.

*Advance:* A locally-acting cell signaling molecule, called *Wnt-4*, has now been shown, based on the effect of mutations in this gene, to be crucial for female sexual development. At birth, sexual development in male mice with a mutation in *Wnt-4* is normal; however, *Wnt-4* mutant females are masculinized--the Müllerian duct is absent while the Wolffian duct continues to develop. *Wnt-4* is initially required in both sexes for formation of the Müllerian duct; in the normal situation, the *Wnt-4* signal in the developing ovary appears to suppress the development of cells in the testis associated with testosterone production (Leydig cells). However, *Wnt-4* mutant females activate testosterone biosynthesis. The *Wnt-4* signal may also be required for maintenance of the female germ cell line. Thus, the establishment of both genders is under the control of both local and general body (systemic) signals.

*Implications:* The understanding of human development at the cellular level depends on tracing cell differentiation from the initial embryonic stage. Differentiation of the gonads into male and female is a fundamental case in point, aside from the general interest in how men and women become differentiated by gender. This advance is an important contribution to understanding the molecular interactions underlying development of the female, and may provide essential information for understanding disease conditions as well.

Vainio S, et al., Female Development in Mammals is Regulated by Wnt-4 Signaling. *Nature* 1999; 397: 405-409.

## **New Therapeutic Approach for Inborn Metabolic Errors**

*Background:* Glycosphingolipids (GSLs) are found in the outer layer of the plasma membrane in virtually all vertebrate cells. Although these glycosylated lipids are believed to participate in several important cellular processes--such as cell to cell interactions--their precise roles are only beginning to be clarified. When the cellular machinery responsible for the degradation of these lipids is defective, the GSLs accumulate in the cell and trigger a pathway leading, in most cases, to nerve cell destruction and severe disease. Examples of GSL storage diseases include Tay-Sachs disease, Sandhoff disease, and Gaucher's disease. In each of these diseases, accumulation of GSL is unique to the disease. Treatments are limited and not effective for the majority of the disorders. An emerging strategy for the treatment of the GSL disorders is called substrate deprivation therapy.<sup>6</sup> In this approach, a specific inhibitor of GSL synthesis is used to reduce accumulation and storage of a particular GSL by lowering the rate at which the protein is made to more closely match the defective rate of protein degradation. Using this approach, a single inhibitor may be used to effectively treat several of the disorders. For example, NIH researchers have demonstrated the prevention of lysosomal storage in Tay Sachs mice treated with the inhibitor NB-DNJ. This agent blocks a particular step in the synthesis of glucose-based GSLs and could therefore be used to treat all storage diseases with defects in the degradation of glucose-based GSLs. Results to date suggest that this strategy may work, but work still remains. This includes working out mechanisms of expected clinical benefit, and potential harmful effects of GSL depletion, and identification of those diseases most suited for therapy.

*Advance:* NIH investigators have addressed these concerns by creating a mouse model of substrate deprivation therapy for a glycosphingolipid storage disease. Mice with Sandhoff's disease, which accumulate GSLs abnormally, were bred with mice that were blocked in their synthesis of GSLs. These mice had simultaneous defects in synthesis and degradation of GSLs and no longer accumulated GSLs, had a much longer lifespan, and had improved neurologic function, though function was not restored to the same level as that of control mice. This additional model supplements the approach taken with treatment of Tay-Sachs disease (above).

*Implications:* The genetic model validates the concept that substrate deprivation therapy can be highly effective in eliminating abnormal lipid accumulation in GSL disorders. Although the model illustrated potential limitations, overall the results advance substrate deprivation therapy as a new treatment modality for the GSL diseases. [secondary B treatment and prevention]

Liu Y, et al., A Genetic Model of Substrate Deprivation Therapy for a Glycosphingolipid Storage Disorder. Journal of Clinical Investigation (1999); 103: 497-505.

## **The Role of Breast Cancer Gene (BRCA 1) Mutation in the Development of Breast Cancer**

*Background:* Breast cancer is the most common cancer in women, with about one in nine women developing this cancer in their lifetime. An estimated 175,000 women will be diagnosed with breast cancer in 1999; about 43,000 will die. Mutations in a gene called BRCA1 are found in ninety percent of all women who have inherited both breast and ovarian cancer, and in about fifty percent of women with inherited breast cancer alone. Until recently, it was unknown how alterations in BRCA1 affected the timing and process of tumor development.

*Advance:* A team of NIH researchers has uncovered the role of two key genes implicated in the development of inherited forms of breast cancer, unveiling a model of tumor formation that holds potential for studying breast cancer in women. First, to uncover the function of BRCA1, researchers bred mice to contain mutations in BRCA1 genes in mammary tissues only, and only at specific points in development. Studies of the resulting mice allowed researchers to build an accurate model of the mechanism by which BRCA1 mutations lead to the formation of tumors. When BRCA1 alone was deleted from mammary glands, five of twenty three mice developed tumors over one year. The tumors showed rearrangements of chromosomes where p53, another gene important in tumor formation, is located. To further determine the role of p53 in breast cancer, researchers introduced a copy of a mutated p53 gene into the mice. When the two mutations were present together, eight of eleven mice formed tumors, a ninety percent increase. Tumors in these mice also grew faster. This research shows that mutations in BRCA1 create an environment in which genes such as p53 mutate more readily. In this case, p53 is believed to be the Gatekeeper. When you lose p53, the tumor grows, providing direct evidence that p53 is involved in BRCA1-associated tumor formation.

*Implications:* Animal models are vital to our understanding of human disease. This is the first such model that shows cancer development similar to the development of breast cancer in women. It will be an important tool for future work on tumors and their progression. This model also provides a route for testing drugs to prevent tumors in humans, as well as to test the role of environmental agents, such as radiation or environmental estrogen in tumor growth. [secondary B treatment]

Xu X, et al., Conditional Mutation of Brca1 in Mammary Epithelial Cells Results in Blunted Ductal Morphogenesis and Tumour Formation. Nature Genetics (1999) 22: 37-43.

## Progress in Zebrafish Research

*Background:* The zebrafish (*Danio rerio*) is recognized as a valuable model for the understanding of human genes, human development, and human diseases. The NIH undertook a Zebrafish Genome Initiative as a result of planning in 1977, with oversight by the Trans-NIH Zebrafish Coordinating Committee. Thirteen institutes contributed funds for an RFA, resulting in awards to five research institutions in late 1998 and early 1999.

*Advance:* The NIH is supporting the establishment of a zebrafish stock center. This central source for the fish is critical for the field. Plans are underway for integrated informatics for zebrafish genes and mutants and for further interfacing with other genome databases. Other progress in the overall program: (1) Development of a high resolution map. This will refine a newly published map showing 2000 markers of gene sites by adding 5200 additional markers, over 3 years. To date they have added 200 new markers, and have submitted 3300 new sequence-tagged sites (markers) to the national database for public use. (2) Generation of sites of active genetic expression (Aexpressed sequence tags®, or ESTs), to be used for candidate genes for various mutants. They project identification of from 30,000 to 35,000 zebrafish genes. The goal is to sequence 100,000 of the ESTs. They will compare gene-based zebrafish maps to the human genome. To date they are generating ESTs at about 700 per week, and have deposited over 16,000 EST sequences in a database for public use. (3) Collaboration to localize zebrafish genes on an integrated genetic map showing linkages among sites. They are placing 3000 ESTs on this (meiotic) map; 303 markers have been assigned preliminary map positions. (4) Creation of deletion mutants of the entire genome. This will provide a resource for looking at gene mapping and gene function.

*Implications:* Zebrafish genome studies will provide clues to processes of human development, mutations, and diseases. For example, the zebrafish gene (Asauternes®) causing anemia is the same as the defective human gene causing sideroblastic anemia. Another mutant (Ayquem®) has a form of porphyria, with photosensitive red blood cells. Thus, work on mutant fish may help to identify new therapies for patients with defects in these enzymes. Many of the key proteins that determine the function of cells are conserved across life forms.

Brownlie A et al, Positional Cloning of the Zebrafish Sauternes Gene: A Model for Congenital Sideroblastic Anemia. Nature Genetics 1998 Nov; 20:244-50.

Shimoda N et al., Zebrafish Genetic Map With 2000 Microsatellite Markers. Genomics 1999;58:219-32.

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Reports of the Trans-NIH Zebrafish Coordinating Committee.

## Regulation of Genetic Expression During Blood Formation

*Background:* Hemoglobin (globin, as used here), is a protein which is responsible for carrying oxygen in red blood cells. There are several globin genes which are expressed at different stages during development and are regulated by an adjacent region known as the locus control region (LCR). This regulatory region occurs in both the mouse and the human and its genetic (nucleic acid) sequence appears to be conserved across species.

*Advance:* Intramural scientists have sequenced the regions surrounding the beta-globin region of both the mouse and human gene and found that these regions, like the LCRs, are similar. Researchers have also identified regulatory sequences preceding the globin genes which are essential for their proper activation (gene expression). In addition, they have discovered that the beta-globin LCRs for both mouse and human are located within a pattern or complex of odorant receptor genes. The odorant receptors, essential for the sense of smell, are expressed in the tissue lining the nose. In the chicken, a gene for the folic acid receptor has been identified which is separated from the beta-globin region by a sequence called an insulator element. Insulators function in gene expression by setting the boundaries of genetic regions. In addition, an odorant receptor gene was also identified in chicken and found to be located next to the beta-globin locus. Expression studies involving this region have shown that activation of the folic acid receptor comes before expression of beta-globin during the development of the red blood cells and that the two genes are independently regulated. It is believed that the insulator element might be important for the proper regulation of these two genes.

*Implication:* These results demonstrate that the mouse and human beta-globin regions are located within a complex of odorant receptor genes. These results suggest that there is a possible role for the beta-globin LCR in the regulation of genes controlling the sense of smell. The extensive cross-species conservation of the beta-globin LCR sheds light on the general basis of blood formation in animals, and validates the study of animal models to further our understanding of human function and disease. The studies on insulators demonstrate a mechanism for functional separation of adjacent genes and have led to the creation of a model of the insulator as a gene sequence boundary in blood forming cells. It is possible that the insulator elements might be important for the proper regulation of two adjacent genes which are activated at different stages during the developmental process in the blood forming cells. [secondary B treatment]

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Prioleau MN, Nony P, Simpson M, and Felsenfeld G, An insulator element and condensed chromatin region separate the chicken beta-globin locus from an independently regulated erythroid-specific folate receptor gene. EMBO J 1999 Jul 15;18(14):4035-48.

## Proteins as Genetic Material in Human Disease

*Background:* Several neurodegenerative diseases are characterized by the accumulation of abnormal forms of normal protein. Some of the best understood members of this group are the spongiform encephalopathies which include scrapie of sheep, kuru, Creutzfeldt-Jakob disease, fatal familial insomnia of man, and Mad Cow disease. The underlying disease process is the same for all the spongiform encephalopathies and is thought to be caused by prions. A prion is an infectious defective form of a protein which has lost its normal function, but has acquired the ability to convert the normal form of the protein into this defective form. Once a protein has undergone this change, it will change all its interacting proteins to the altered form. These proteins, or prions, will become infectious if they can be transmitted from one organism to another. NIH researchers, based on the fulfillment of three genetic criteria, have proposed that two non-Mendelian elements of yeast are infectious proteins similar to the prion protein implicated in diseases of mammals and have reported additional biochemical and cell biological evidence to further support this theory. Researchers demonstrated spontaneous generation of one particular yeast prion protein and showed that this protein can be produced in a yeast strain which could not have had the element before. Researchers are now working towards demonstrating that a fusion protein consisting of a mammalian prion protein with part of a yeast normal protein can mediate an infectious process in yeast.

*Advance:* In Alzheimer's disease, a protein fragment of a larger precursor protein forms amyloid. Accumulation of the amyloid protein (amyloidosis) in the brain has been implicated in the disease process. Researchers have demonstrated that a normal yeast protein is capable of inducing amyloid formation only when it contains a prion domain peptide, implying that this yeast prion protein is acting as an infectious agent.

*Implication:* These data strengthen the argument for the prion model and shed light on the interactions required for inducing the alterations from the normal to the prion state. This work may lead to the discovery of mechanisms for amyloid formation in Alzheimer's disease and provides a method to search for other prion proteins that may lead other forms of transmissible degenerative neurological diseases. This is a vast new field of research crucial to the understanding of and future intervention in degenerative neurological disease processes. [secondary B prevention]

Wickner RB, Edskes HK, Maddelein ML, Taylor KL, and Moriyama H, Prions of yeast and fungi. Proteins as genetic material. J Biol Chem 1999 Jan 8;274(2):555-8

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Edskes HK, Gray VT, and Wickner RB, *The [URE3] prion is an aggregated form of Ure2p that can be cured by overexpression of Ure2p fragments.* Proc Natl Acad Sci U S A 1999; 96(4):1498-503

## **Appetite-Regulating Protein Implicated in Alcohol Consumption**

*Background:* Appetite is more than simple hunger; it is a complex network of biochemical interactions that respond to different drives, with the brain acting as command center. Neuropeptide Y (NPY) is the most widely distributed peptide (a protein component) in the brain and is the most potent of the known appetite-stimulating peptides. NPY injections into certain parts of the brain not only promote eating, but also reduce anxiety-related behaviors, reduce seizures, and increase sedative effects of some drugs.

Scientists have found that NPY, which is of considerable interest to obesity and diabetes research because of its crucial role in regulating appetite for food, also plays a role in regulating appetite for alcohol -- and presents a paradox. While a large body of research has demonstrated that *high* levels of NPY dramatically increase food intake, a new study reveals that *low* levels of NPY have the same effect on alcohol intake. The study was the first to test effects of NPY on appetite for alcohol in mice genetically altered not to produce NPY or to produce more than is normal. Changes in a single NPY-related gene resulted in changes in the mice's drinking behavior. Previous studies altered NPY levels not genetically but pharmacologically, via brain injections.

The complexity of the biochemistry of appetite regulation is becoming increasingly evident, resulting in a profusion of research. Scientists now are in the early stages of investigating whether appetite follows a common biochemical pathway for food, alcohol, water, drugs, and other substances. Among the possibilities raised by the new finding is that this hypothetical pathway diverges for food and alcohol. If this is the case, identifying these pathways will help scientists search for ways of therapeutically altering them.

*Advance:* Alcohol researchers eliminated the NPY gene in embryonic mice, rendering them unable to produce the protein as they grew. The researchers then demonstrated that NPY-deficient mice consume significantly more alcohol than do unaltered mice. Conversely, when researchers genetically altered mice to produce *excess* NPY, the mice significantly reduced their alcohol intake. But, unlike animals whose NPY levels were altered with injections, mice with genetically induced NPY alterations did not eat abnormally. In addition, researchers found that animals lacking the NPY gene were more resistant to alcohol's effects. High resistance to alcohol's effects is associated with heavier alcohol-drinking, in both animals and humans, while low resistance is associated with less drinking.

*Implications:* Appetite regulation has implications for serious health problems, such as diabetes and alcoholism. It is unlikely that NPY alone, or any single substance, causes alcoholism, which affects 14 million American adults and costs U.S. society \$166 billion each year. Rather, this finding adds to the understanding of biological mechanisms that underlie alcohol use and provides potential clues about drinking-related phenomena in humans. Scientists are studying how these mechanisms interact to form circuits in the nervous system that regulate behaviors, such as heavy drinking, that are harmful to health and have potential for interventions.

Thiele TE, Marsh DJ, Ste. Marie L, Bernstein IL, Palmiter RD. Ethanol Consumption and Resistance Are Inversely Related to Neuropeptide Y Levels. Nature, 396:366-369, November 1998.

## **Adolescents May Be Vulnerable to Some Types of Alcohol-Induced Memory Impairment**

*Background:* Intuitively, it might seem that people who can hold their liquor are at low risk for alcohol-related problems. But the opposite is true: these people are at higher risk, because their tolerance for alcohol allows them to drink more. Even while they drink larger amounts of alcohol with seeming equanimity, compared to other people, they are exposing themselves in larger degree to the physiological changes that lead to trouble -- adaptations in the brain that require more and more alcohol in the future in order to feel the same pleasurable effects and changes in the hard-wiring of the nervous system, for example.

Some adolescents can tolerate large amounts of alcohol, previous studies suggest. Researchers have found that, compared to adults, adolescents do not feel as readily the uncomfortable sensations, such as sluggishness and nausea, that alcohol causes. Their nervous systems are not as easily dampened by alcohol. This means they can drink more, because it does not make them feel badly as quickly.

Researchers are concerned by this capacity for drinking among some young people. Adolescents actually do have a disproportionately high rate of drinking, and this phenomenon occurs at a biologically and behaviorally vulnerable time of life. For example, adolescents, unlike adults, are still forming connections between nerve cells that play a role in memory, and toxic substances may damage the development of these connections. Does alcohol permanently alter molecular or gene-related changes that normally take place in adolescence, including changes in the nervous system? If so, what are these harmful changes? Can they be therapeutically altered? These are among the questions researchers are asking as part of an initiative to identify alcohol-induced physiological and behavioral changes unique to adolescents.

Scientists can assess these types of changes by measuring different alcohol-induced behaviors, then relating the behaviors to the anatomical structures and biological mechanisms that underlie them. One behavior affected by alcohol is spatial memory; that is, memory of location of objects in the environment and how to get to them. Spatial memory is known to be processed by a brain structure called the hippocampus.

*Advance:* For the first time, researchers found that alcohol significantly impaired spatial-memory acquisition in adolescent, but not adult, rats in a water-maze test. In a separate experiment, alcohol did not impair acquisition of nonspatial memory in either group performing a task in the water maze.

*Implications:* The advance described here suggests that adolescents are at risk of diminishing their spatial-learning abilities if they drink excessively. Potential consequences of these findings, should they be found also to apply to humans, include impaired cognitive function -- knowing, awareness, and judgment. Whether or not such impairments occur in human adolescents and, if so, whether or not they can be prevented or reversed remain under study. [secondary prevention]

Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS: Differential Effects of Ethanol on Memory in Adolescent and Adult Rats. Alcohol: Clinical and Experimental Research, 22(2):416-421, April 1998.

## **Scientists Discover Gene Mutations Defining New Group of Inflammatory Diseases**

*Background:* NIH intramural scientists had previously led an international consortium in cloning the gene for familial Mediterranean fever (FMF), a hereditary disorder of fever and inflammation common among people of Jewish, Arab, Armenian, and Turkish ancestry. After the FMF gene was identified, it became clear that some families with the characteristic periodic fevers did not have FMF mutations.

*Advance:* NIH intramural researchers and their collaborators from around the world have discovered genetic mutations on chromosome 12 underlying a newly recognized group of inherited inflammatory disorders that includes familial Hibernian Fever. The disorders, collectively known as TRAPS (TNF Receptor-Associated Periodic Syndrome), are characterized by long, dramatic, episodes of high fever, severe pain in the abdomen, chest, or joints; skin rash; and inflammation in or around the eyes. Some patients also develop amyloidosis, a potentially fatal disease in which a blood protein is deposited in vital organs. The mutations involve a cell surface receptor for the inflammatory protein tumor necrosis factor. The receptor mutations are thought to predispose individuals to severe inflammation triggered by emotional stress, minor trauma or for no apparent reason.

*Implications:* These results are very important in helping us further understand the role of the TNF pathway in disease, and they mark the first time that TNF receptor mutations have been tied to an inherited disease. This discovery may lead to additional treatments, targeted at the cellular level, for many immune-related and inflammatory disorders. Researchers are now studying the potential usefulness of synthetic forms of the TNF receptor in suppressing inflammation in affected patients. Fortuitously, a drug recently approved for the treatment of rheumatoid arthritis is in fact the shed form of a related TNF receptor. Researchers will now determine the potential usefulness of this drug in treating TRAPS. Currently, many patients are treated with high doses of steroids, which can have serious side effects and are not completely effective. Researchers are hopeful that the discovery of TNFR1 mutations will help TRAPS patients. [secondary B treatment ]

McDermott, et al. Germline mutations in the extracellular domains of the 55 kDa TNF Receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* 97: 133-144, 1999

### **Inbred Mouse Strains Yield Clues to the Genetics of Bone Density**

*Background:* Much evidence indicates that an individual's genetic makeup influences peak bone density. High peak bone density, in turn, can protect against the risk of debilitating fractures because an individual's bone mass declines with age. It has become clear that a number of genes are involved in the determination of bone density, posing a complex problem in quantitative genetics.

Fortunately, however, many aspects of bone biology have been found to be similar in humans and mice, and many mouse genes are similar to their human counterparts. Furthermore, geneticists have managed to create a number of inbred mouse strains in which each mouse is essentially genetically identical to its parents.

*Advance:* Using pairs of inbred mice with different bone densities, researchers have found that cells that break down bone, osteoclasts, are more numerous in the low-density strain. (Earlier studies showed that bone-forming osteoblasts are more numerous in a high-density strain.)

*Implications:* If investigators can identify the genes responsible for differences in peak bone density between inbred mouse strains, it will be possible to test the influence of the corresponding genes in human populations.

Linkhart TA, Linkhart SG, Kodama Y, Farley JR, Dimai HP, Wright KR, Wergedal JE, Sheng M, Beamer WG, Donahue LR, Rosen CJ, Baylink DJ: Osteoclast formation in bone marrow cultures from two inbred strains of mice with different bone densities. J Bone Miner Res 14: 39-46, 1999.

### **Febrile Seizures Modify Brain Excitability**

*Background:* Febrile, that is, fever-related, seizures are very common, occurring in 3-5% of infants and young children, typically between the ages of 6 months and 5 years. Simple febrile seizures are usually quite short (less than 5 minutes) and occur when the fever is just starting. They are very frightening to parents and often lead to emergency room visits and hospitalization, but there has been considerable controversy about whether febrile seizures cause epilepsy later in life. Although epidemiological studies looking at large numbers of normal children with febrile seizures have not found that these children are more likely to develop epilepsy, many adults undergoing surgery for intractable temporal lobe epilepsy report a history of prolonged (greater than 15 minutes) febrile convulsions. If febrile seizures are indeed benign, children do not have to be put on medication. If, on the other hand febrile seizures have long-term adverse effects on the brain, medications to prevent seizures or their consequences may be warranted.

*Advance:* To address the question, investigators induced prolonged (about 20 minutes) seizures in young rat pups by elevating their body temperature similar to that of children with a high fever. They found long lasting changes in the electrical activity of specific types of nerve cells in the hippocampus, an area of the brain critical in epilepsy.

*Implication:* The investigators are now studying how the brain responds to this change in nerve cell function and looking into whether their findings can be applied to humans. They believe that febrile seizures change the way some brain cells function, which by itself does not cause epilepsy, but may decrease the protection the brain normally has against seizures. Although definitive answers to the questions of febrile seizures are still elusive, this new avenue of investigation should provide new insights into how seizures affect the brain that may be relevant to many forms of epilepsy. [secondary B treatment]

Chen K, Baram T, Soltesz I: Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. Nature Medicine 5:888-894, 1999.

## Understanding the Potential of Neural Stem Cells

*Background:* Research on stem cells has stolen headlines and excited the public interest like few discoveries in biomedicine. News reports raise the possibility of repair and replacement of diseased organs that had previously been beyond the scope of medical or surgical intervention. The isolation of human stem cells could, in theory, allow scientists to cultivate any of the body's tissues for repair and replacement in disease states. Realizing this potential will not be easy. Because the brain and spinal cord have a very limited ability to repair themselves, for no area of medicine is the promise of stem cell therapy more tantalizing than for disorders of the nervous system. However, the task is particularly daunting for the brain and spinal cord because of the variety of cell types, the complexity of cell connections, and the range of functions subserved by the existing circuitry within the mature nervous system. In spite of these obstacles, promising results from research raise hope that stem cells may be used to treat disorders in the brain.

*Advances:* Three recent accomplishments are particularly relevant.

- \$ Demonstration that neurogenesis, that is the birth of new nerve cells, continues throughout life. Scientists have recently found that stem cells continue to generate new nerve cells in some parts of the brain even in 60 year old people.
- \$ Demonstration that neurogenesis in the adult nervous system is a dynamic process, and responds to both internal and environmental factors. Activities such as exercise, circulating hormones, learning, and an enriched environment can promote neurogenesis, while adverse conditions such as stress have a negative impact on the production of new nerve cells in the brain.
- \$ In rodent models, transplantation of stem and multipotent progenitor cells have been able to replace cells and restore deficits in the diseased and damaged brain. Multipotent progenitor cells are not as versatile as stem cells, but can multiply and form several, though not all, cell types of the brain.

*Implications:* Before stem cells can be used therapeutically, the factors that influence their growth and specialization must be thoroughly understood. These cells have the capacity to form both nerve cells and glia, the supporting cells of the nervous system. Glial cells are crucial to proper function of the nervous system and are affected in many disorders, such as multiple sclerosis, and by trauma. The fact that stem cell division and differentiation can be influenced by sensory input or circulating hormones suggests that concurrent drugs or rehabilitation may facilitate or enhance their eventual usefulness; these possibilities can be tested in animal models and the results used to guide clinical applications.

Although stem cells may have eventual applications to human disease, their very nature of proliferation, responsiveness, and complex specialization must sound a note of concern. Undifferentiated cells or the factors that influence cells to replicate or migrate, have a "dark side." Could cancer be the genie in the bottle of magic elixir? Before patients are exposed to implantation procedures, scientists must reveal the potential risks and benefits of the cells to be used. [secondary B treatment]

*FY99 NIH GPRA Research Program Outcomes*

Yandava BD, Billingham LL, and Snyder EY: "Global" cell replacement is feasible via neural stem cell transplantation: Evidence from the dysmyelinated *shiverer* mouse brain. Proc Natl Acad Sci USA 96:7029-7034, 1999.

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ: Learning enhances adult neurogenesis in the hippocampal formation. Nature Neuro 3:260-265, 1999.

Van Praag H, Kempermann G, Gage FH: Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuro 3:266-270, 1999.

## Gene for Narcolepsy Discovered

*Background:* Narcolepsy is a serious brain disorder that affects sleep in a dramatic way. Usually beginning in adolescence, narcolepsy's symptoms include extreme daytime sleepiness and sleep paralysis—a frightening inability to move shortly after awakening or shortly after dozing off. For many people the most serious symptom is sudden episodes of muscle weakness called cataplexy. In extreme cases a person may abruptly collapse to the floor completely paralyzed for several minutes. Cataplexy can occur at any time, but is often triggered by strong emotions such as anger, joy, surprise or laughing. Narcolepsy ruins lives. It impairs job and school performance, predisposes people to have accidents, and can seriously impair social life. An average of 14 years pass before the disorder is properly diagnosed, and, because little is known about what causes narcolepsy, treatment is limited to general nervous system stimulants.

*Advance:* After a decade long search, a team of scientists has discovered the gene defect that causes an inherited form of narcolepsy in dogs that closely mimics the disease in people. Dogs are one of the few animals that are affected by the disorder, and in certain Dobermans and Labradors narcolepsy is caused by a single gene that must be inherited from both parents, which simplified the search for the gene. Importantly, the gene provides an important clue to the cause of the disorder that may lead to more effective treatment. The gene codes for a receptor, that is the cell's sensor, for a chemical messenger called hypocretin. Hypocretin plays an important role in sleep regulation. The same gene exists in humans and researchers plan to look for defective versions in people with narcolepsy.

*Implications:* About 1 person in 2000 is affected by narcolepsy to some degree. Like many neurological disorders, some people inherit the disease and others do not, but understanding the inherited form should help understand what causes all types of narcolepsy. In time, this discovery may also shed light on two of the biggest mysteries in sleep research: how and why we sleep. Unraveling the mysteries of sleep will ultimately have implications for the large number of people who suffer from many types of sleep disorders. [secondary B treatment]

Ling L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, deJong P, Nishino S, and Mignot E: The Sleep Disorder Canine Narcolepsy is Caused by a Mutation in the *Hypocretin (Orexin) Receptor 2* Gene. Cell 98: 365-376, 1999.

## Understanding a Retinal Degenerative Disease

*Background:* A molecule called the rim protein (RmP) of the retinal rod photoreceptor cell is a component of the outer segment discs. RmP was discovered in 1978 but no function could be assigned to it for many years. Recently, this protein has been associated with a specific eye disease--Stargardt's disease or fundus flavimaculatus. The gene for Stargardt's disease has been identified and shown to produce a transporter molecule that moves substances into and out of the cell. While an interesting and important finding, the exact function of this protein in the eye was unclear. Where exactly was this protein located in the eye and what did it do? The transporter that is defective in Stargardt's disease belongs to a very large class of membrane proteins called the ATP-binding cassette (ABC) transporter superfamily. These transporters use a high energy compound, ATP, to power movement of molecules across membranes. Studies on the cellular location of the ABC rim (ABCR) protein within the visual cells showed it to be located to the interior side of the rod outer segment disc membrane. This narrowed the range of plausible functions for ABCR and the corresponding range of pathogenic mechanisms responsible for Stargardt's disease. Scientists speculated that ABCR may be involved in the movement of retinoids--vitamin A and its analogs. Retinoids are essential for the processes that initiate vision.

*Advance:* Recent work has demonstrated that the retinal rod rim protein (RmP) is identical with the ABC transporter responsible for Stargardt's disease--ABCR. First researchers set out to discover what molecules in the retinal cells might regulate the transport activity of ABCR in order to gain more information on its specific function. It was known that the ABCR binds ATP. So it was necessary to find a compound or compounds that caused the splitting of ATP by ATPase and resulted in loss of ABCR transport activity as ATP binding decreased. Analogs of vitamin A, particularly retinal, turned out to be potent stimulators of ATP hydrolysis and thus regulators of ABCR. This implicated the vitamin A cycle in the function of ABCR. The second approach used knockout mice, which are useful tools for uncovering function. If a gene can be knocked out its function may be discovered. In this case the ABCR gene knock out resulted in altered retinoid metabolism. Specifically, ABCR acts to flip and thus move molecules from one side of the rod disc membrane to the other. In Stargardt's disease a pigment called lipofuscin accumulates in the pigmented epithelium. Lipofuscin is composed mainly of a molecule called A2E. ABCR knockout mice had much higher levels of A2E than normal. So it appears that if the ABCR transporter is functioning improperly, A2E accumulates in the photoreceptor discs, leading to subsequent dysfunction.

*Implications:* A better understanding of the pathogenesis of photoreceptor cell dysfunction may ultimately help prevent or effectively treat retinal degenerative diseases.

Weng J, Mata NL, Sassan MA, Tzekov RT, Birch DG, and Travis GH: Insights into the function of rim protein in photoreceptors and etiology of Stargardt's Disease from the phenotype in abcr knockout mice. *Cell* 98:13-23, 1999.

## **Retina-specific Gene Causes Autosomal Dominant Retinitis Pigmentosa**

*Background:* Inherited retinal degenerations, such as retinitis pigmentosa (RP), are an important cause of visual impairment in children and young adults. Clinically, these diseases are characterized by night blindness and progressive vision loss. Several genes have been identified for several forms of autosomal recessive (AR), autosomal dominant (AD), and X-linked (XL) retinal disease, but the genes responsible for many types of RP are still not identified.

*Advance:* Mapping studies in families with AD-RP located the first well-characterized AD-RP disease locus, called RP1 within the chromosome 8. All families studied had similar clinical characteristics with relatively late onset of night blindness and slow progression.

Two prior advances helped in the search for the gene. One was that it was known that in the mouse, chromosome 4 had a similar DNA base sequence, called synteny, to the human sequence in chromosome 8. This region of the mouse chromosome had been shown to contain a dominant retinal degeneration locus called RD4. This locus contained a small piece of DNA that had become turned backwards on itself, or inverted, and was re-inserted back into the genome. Thus, RP4 was caused by a DNA inversion. This was a clue that this region was an important one. Second, small unique stretches of DNA called expressed sequence tag (EST) sites which can serve as guideposts, were available in both RP1 from human and RP4 from mouse.

Using ESTs from both the mouse and human as location markers on the genome, researchers were able to sequence sections of mouse and human DNA that might contain a candidate for a disease gene. They found a mutation which produced a truncated, dysfunctional protein. DNA from affected members of one family was screened for this mutation. In all cases the mutation was found in affected individuals but not in normal individuals. The second family was also screened for the mutation with positive results. DNA sequencing from the third family revealed a different mutation, predicting a different shortened or truncated protein.

*Implications:* The function of the protein product identified in this study and its role in the disease process remains unknown. Database searches suggest the RP1 gene product may be a protein kinase. It appears to share similarities with a protein called doublecortin. This suggests it may have a role in development or maintenance of the neural retina.

Sullivan LS, Heckenlively JR, Bowne SJ., Zuo J, Hide WA, Gal A, Denton M, Inglehearn CF, Blanton SH, and Daiger SP: Mutations in a novel retina-specific gene cause autosomal dominant retinitis pigmentosa. Nature Genetics 22:1-5, 1999.

## **New Findings Link Nitric Oxide to Nerve Cell Damage in Glaucoma**

*Background:* Glaucoma is a family of disorders that are characterized by a distinct pattern of optic nerve loss and diminished peripheral vision, and in many cases, progresses to total blindness. The most common form of the disease, primary open angle glaucoma (POAG), affects 3 million, mostly older, Americans and is the leading cause of blindness among African-Americans. About 120,000 Americans are blind as a result of glaucoma. All glaucomas share a distinct type of progressive damage to the optic nerve, the structure that transmits information from the retina to the brain. In this disease, damage to the axons of retinal nerve cells sets up a degenerative process whose end-result is vision loss. Elevated intraocular pressure is frequently associated with glaucoma and explanations for how axons become damaged are usually based on the mechanical effects of elevated intraocular pressure. However, optic nerve damage can occur without abnormally high pressures and conversely, elevated pressure does not necessarily lead to optic nerve damage. This has led scientists to seek a unifying explanation for optic nerve degeneration that includes elevated intraocular pressure as one of many possible causes. Present treatments are based on reducing intraocular pressure; however, treating the actual cause of vision loss by preventing degeneration of the axons is the ultimate goal. Discovering the basis of optic nerve degeneration is essential for the development of the next generation of glaucoma drugs, neuroprotective agents.

*Advance:* Scientists now have evidence that the molecule nitric oxide (NO), is directly involved in mediating the degeneration of axons in the optic nerve head. Since its discovery, NO has been shown to have a number of varied physiological affects including mediating muscle relaxation, vasodilatation, and transmission of neural impulses. It is best known as the basis for the development of the drug, Viagra<sup>1</sup>. Using a rat model of glaucoma in which intraocular pressure is artificially elevated, scientists first found that optic nerve pathology mimics that of humans. Treating these rats with a compound that inhibits the production of nitric oxide protected the axons of these rats even though intraocular pressure remained higher than normal.

*Implications:* There is a growing realization that viewing glaucoma as solely a problem of intraocular pressure is not adequate. Even though glaucoma has been likened to other degenerative diseases such as Alzheimer's, research into plausible degenerative pathways is just in its initial stages. This report is the first in which protection of the optic nerve was separated from reducing intraocular pressure. By showing a connection between the production of a physiological agent, nitric oxide, and damage to retinal nerve cell axons, the results in this paper are the first to suggest a pathway. Lastly, these findings begin the process of identifying and developing neuroprotective agents as a new class of glaucoma drugs. [secondary B treatment]

Neufeld AH, Sawada A, and Becker B: Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. Proc Natl Acad Sci USA 96:9944-48,1999.

## **New Clues to How the Lens Forms and Maintains Its Structure**

*Background:* The lens of the eye is an ellipsoid-shaped structure that helps focus images onto the retina. In contrast to the cellular and molecular complexities present in most other tissues, the lens consists of only two cell types: epithelial cells, which form a single layer on the outer surface, and mature fiber cells, which fill the internal cavity. This structure helps make the lens transparent, a characteristic that is critical to its function. Fiber cells are made from the lens epithelial cells in a process called differentiation. In order to maintain the number of epithelial cells, each epithelial cell replicates itself before it goes on to become a fiber cell. It is this process of replication followed by differentiation that initially forms the lens during development of the embryo. It also maintains lens structure throughout the life-span of the individual. Because lens function is dependent on its structure, replication and differentiation of lens cells are precisely controlled processes of great interest.

*Advance:* The simplicity of the lens structure belies the complexity involved in its formation and maintenance. Lens cell replication and differentiation are interdependent processes whose coordination is dependent on a number of intracellular elements and extracellular signals. Scientists are attempting to identify the individual steps needed for lens formation and to determine the exact nature of the elements and signals needed to coordinate the steps. Especially important are the elements that direct the epithelial cell to stop replicating and start differentiating. The gene product of *Prox1*, a gene which is expressed in a variety of embryonic tissues, appears to be a central regulator of the transition from replication to differentiation. Studies in mice indicate that *Prox1* expression regulates the expression of elements known as cell cycle inhibitors that are required to stop the replication of epithelial cells and begin their differentiation into fiber cells. Absent *Prox1* expression, cell cycle inhibitors are not expressed, throwing the epithelial cells into a spasm of replication without subsequent differentiation and fiber cell formation.

*Implications:* These findings are relevant not only to the developing lens but also to the aging lens. The lens is a continuously growing structure with the processes of replication and differentiation that form the embryonic lens continuing throughout the life of the individual. Anomalies in the functioning of regulatory elements and signals during development result in congenitally malformed lenses that will require surgery to replace. In the adult, malfunctioning of these elements and signals can also be a problem. Many people who undergo cataract surgery develop secondary cataracts, a situation in which residual cells begin replicating and differentiating in an attempt to form a new lens. If this process interferes with vision, laser treatment is required, thereby increasing treatment costs. It is essential to understand the regulation of replication and differentiation of these cells in order to devise new cost-effective preventive strategies.

Wigle JT, Chowdhury K, Gruss P, and Oliver G: *Prox1* function is crucial for mouse lens-fibre elongation. Nature Genetics 21:318-322. 1999.

## **Growth Factor Research May Lead to New Treatments**

*Background:* Pigment epithelium-derived growth factor (PEDF) is a protein found in the healthy eye. PEDF is secreted by the retinal pigment epithelial cells that underlie and nourish the retina. This protein has been found to be potent in extending and protecting nerves from harm and halting excessive blood vessel growth in the retina. The retina is susceptible to a variety of diseases with diverse etiologies that can lead to visual loss or complete blindness, including diabetic retinopathy, retinoblastoma, and macular degeneration. In pursuing development of new treatments for blinding diseases affecting the retina and neurodegenerative diseases affecting the spinal cord and cerebellum, investigators have been trying to understand how PEDF functions.

*Advance:* About a decade ago scientists reported the discovery of PEDF and found it had the ability to cause cells of retinoblastoma tumors to differentiate to resemble non-dividing neurons. Recently, a team of scientists demonstrated that PEDF can transiently delay the death of photoreceptors in mouse models of inherited retinal degenerations. Two teams of scientists have shown that this protein can promote neurite-outgrowth and protect spinal cord motor neurons against natural and induced death using cell culture and animal model systems. Another team of scientists has shown that PEDF has effects on nerves from cerebellum: it can also protect cultured cerebellar granule cells against natural death and chemically-induced insults. Another group of scientists has shown that PEDF can prevent the growth of endothelial cells that form blood vessels. Thus, PEDF behaves as a potent neurotrophic factor for the retina and nerves of the central nervous system, as well as a potent inhibitor of angiogenesis.

*Implications:* Learning how PEDF works may provide information that will contribute to the development of an effective treatment for several neural degenerative diseases (retinitis pigmentosa, macular degeneration, diabetic retinopathy, or even Lou Gehrig's disease, which brings about slow death of motor neurons in the spinal cord). Knowledge of its mechanism of action may teach us about its effectiveness and possible side effects. The availability of a protein delivery system for studying the disease and testing therapeutic strategies will greatly facilitate progress in this important area of research. [secondary B treatment]

Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu HJ, Benedict W, Bouck NP: Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science 285:245-248, 1999.

Houenou LJ, D-Costa AP, Li L, Turgeon VL, Enyadike C, Alberdi E, and Becerra P: Pigment epithelium-derived factor promotes the survival and differentiation of developing spinal motor neurons. J Compar Neurol (in press), 1999.

### **Identification of Modified Forms of $\alpha$ B-Crystallin in Human Cataracts**

*Background:* Aging-related cataract is the leading cause of world blindness. At present there is no non-surgical means of prevention or treatment. Cataract is a multi-factorial disease that occurs when the ocular lens loses transparency, thereby blocking incident light from reaching the sensory elements of the retina. A primary mechanism for loss of transparency involves aggregation of lens proteins that absorb or scatter light. Understanding the mechanisms underlying this aggregation process is critical for development of an effective medical therapy for cataract.

*Advance:* Scientists have identified in certain human cataracts two atypical, modified forms of one of the major lens proteins,  $\alpha$ B-crystallin. The concentration of this protein is also increased significantly in these cataracts.  $\alpha$ B-Crystallin is present in highest abundance in the lens but is also found in many other tissues such as heart and brain. It is one of a family of proteins thought to protect cells against stresses such as heat shock, osmotic stress, and oxidative stress by preventing the aggregation of proteins, an effect that would be particularly beneficial in the lens.

*Implications:* The identification of specific modifications to lens proteins associated with cataract development is a clue toward understanding the etiological basis of the disease. The fact that the modifications are due to a major protein believed to provide protection to the lens increases the possibility that this discovery could be an important step toward the development of an effective cataract treatment. [secondary B treatment]

Jimenez-Asensio J, Clovis CM, Kowalak JA, Duglas-Tabor Y, Datiles MB, Moroni M, Mura U, Rao ChM, Balsubramanian D, Janjani A, and Garland D: An atypical form of  $\alpha$ B-crystallin is present in high concentration in some human cataractous lenses: identification and characterization of aberrant- and C-terminal processing. J Biol Chem (in press).

## **The Dual Role of Interleukin-12 in Regulation of Autoimmune Retinal Disease**

*Background:* Interleukin 12 (IL-12) is an important proinflammatory mediator. It belongs to the group of soluble mediators known as cytokines, i.e., soluble factors produced by cells of the immune system. IL-12 is produced as a first line response to microbial products and other inflammatory stimuli, and has a number of effects on the immune system. It triggers production of other proinflammatory cytokines, and causes the immune response to become a predominantly cellular, rather than an antibody dependent, pathway. While this is important to promote an effective defense against many types of invading microorganisms, it also promotes the types of responses that can result in tissue damage. Scientists have studied the role of IL-12 in autoimmune retinal disease, using the model of experimental autoimmune uveitis (EAU) induced in animals by immunization with retinal proteins. EAU serves as a laboratory equivalent of blinding uveitis in humans, and has been instrumental in the study of basic mechanisms and the development of therapeutic approaches to human uveitis.

*Advance:* Scientists have found that induction IL-12 is necessary for development of EAU in mice that were genetically engineered to be deficient in this cytokine, or that were treated with neutralizing antibodies to IL-12. Detailed studies of the cellular response suggest that IL-12 is involved in fueling progression of established disease. Interestingly, however, mice receiving injections of recombinant IL-12 (or an excess of IL-12) for several days following immunization with a retinal antigen failed to develop disease as expected. Thus, although IL-12 is necessary for development and function of autoimmune lymphocytes, its excess at a critical time point can curtail their development.

*Implications:* These studies have contributed to understanding the role of IL-12 in the regulation of autoimmune retinal disease, and other autoimmune and inflammatory diseases that share similar mechanisms. Because IL-12 appears necessary both for bringing new immune cells into the pool, and for maintenance of their function, targeting IL-12 may be an attractive therapeutic option. Laboratory and clinical scientists are now preparing for a preclinical trial in a primate model of EAU using humanized antibodies to IL-12 as a first step in translating this research to the clinic. [secondary B treatment]

Tarrant, TK, Silver PB, Wahlsten JL, Rizzo LV, Chan CC, Wiggert B, and Caspi RR: Interleukin 12 protects from a T helper type I-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. J Exp Med 189:219-230, 1999.

### **Interferon- $\gamma$ Increases the Severity of Uveitis and Induces Retinal Degenerative Changes in Transgenic Rats**

*Background:* Uveitis is the most common intraocular inflammatory disease and is the cause of significant morbidity and absenteeism in schools and the work place. Although the etiology of uveitis of non-infectious disease origin is unknown, analyses of the eyes of patients with diverse uveitic conditions reveal the presence of the proinflammatory cytokine, interferon- $\gamma$  (IFN $\gamma$ ) and upregulated expression of HLA molecules. Although IFN $\gamma$  has been implicated in the immunopathogenic mechanisms of a number of organ-specific autoimmune diseases, its exact role is still a matter of debate. In experimental mouse models, IFN $\gamma$  has been shown to exacerbate some autoimmune diseases while it confers protection against others, including experimental uveitis. Because of the implications of these findings for therapeutic use of IFN $\gamma$ , scientists have used transgenic rats with targeted expression of IFN $\gamma$  in the eye to study its paracrine effects and investigate whether local production of IFN $\gamma$  confers protection against uveitis in the rat species.

*Advance:* This is the first transgenic rat with constitutive expression of IFN $\gamma$  in the eye. Analysis of these rats reveal that an important consequence of prolonged exposure of ocular cells to IFN $\gamma$ , as may occur during chronic or recurrent uveitis, is the induction of choroidal inflammation, activation of pro-inflammatory genes, increase in the severity of uveitis, formation of retinal folds and ganglion cell layer degeneration. In contrast to its protective systemic effect in the mouse, constitutive secretion of IFN $\gamma$  in the rat eye was found to predispose the eye to development of severe uveitis and induction of retinal degeneration.

*Implication:* Autoimmune diseases include such diseases as multiple sclerosis, lupus, rheumatoid arthritis, diabetes, uveitis and are a major cause of morbidity in the United States. Although these experiments specifically addressed uveitis, these findings are likely applicable to a number of these diseases. The findings also underscore the importance of collecting corroborating evidence in other species before extrapolating to the human condition. The IFN $\gamma$  transgenic rat may also be a unique experimental model for studying etiologic mechanisms of glaucoma and uveitis.

Egwuagu, CE, Sztein, J, Mahdi, RM, Li, W, Chan, CC, Smith, JA, Charukamnoetkanok P and Chepelinsky, AB: Interferon- $\gamma$  (IFN $\gamma$ ) Increases the Severity and Accelerates the Onset of Experimental Autoimmune Uveitis in Transgenic Rats. J Immunol 162: 510-517, 1999.

Egwuagu, CE, Sztein, J, Mahdi, RM, Li, W, Chan, CC, Smith, JA and Chepelinsky, AB: Constitutive Expression of IFN- $\gamma$  in the Eye Exacerbates Anterior Uveitis and Induces Retinal Degenerative Changes in Transgenic Rats. Clinical Immunology 91:196-205, 1999.

## Hair Cell Differentiation and Specification

*Background:* In humans, the loss of auditory sensory cells (hair cells) results in deafness and hearing impairment. These sensory hair cells are established early in development and following injury they are not replaced. In birds, by contrast, proliferation to produce hair cells is observed following injury. Thus, understanding how sensory cells are generated in the mammalian organ of Corti may someday lead to treatment for hearing impairment. Sensory hair cells are formed during development and current approaches are examining the molecular and cellular switches that direct progenitor cells to differentiate to their final state as sensory hair cells or other type of cells. Many of the factors that signal differentiation, as well as why these mechanisms fail to operate in regeneration of adult mammalian hair cells, are unknown.

*Advance:* Previous results suggest that determination of cell identity in the cochlea occurs by inhibitory interactions between adjacent progenitor cells. Investigators have recently provided direct evidence for Notch-mediated lateral inhibition, a molecular mechanism known to be involved in determining cell fate, in the mammalian system. Notch-mediated lateral inhibition regulates the number of progenitor cells that develop as hair cells, providing important clues into the molecular processes limiting hair cell development. A separate team of NIH-supported investigations have shown that another signaling molecule, *Math1*, is essential for the formation of hair cells. *Math1*, a mouse homolog of the *Drosophila* proneural gene *atonal*, directs precursor cells in the inner ear to become hair cells. Mice lacking this gene failed to produce hair cells, showing that *Math1* is required for hair cell development and differentiation.

*Implications:* *Math1* is the first gene shown to be required for the specification of hair cells. These two findings provide novel insight into the molecular signaling effects involved in hair cell differentiation and specification and are critical to future clinical application.

Lanford PJ et al: Notch signalling pathway mediates hair cell development in mammalian cochlea. [Nature Genetics](#) 21: 289-292, 1999.

Bermingham NA et al: *Math1*: An essential gene for the generation of inner ear hair cells. [Science](#) 284(183): 837-841, 1999.

### **A Novel Calcium Response in Hair Cells**

*Background:* Hair cells are highly specialized cells in the inner ear that convert sound or motion into neural impulses. Damage to hair cells is often the cause of hearing and balance disorders. Hair cells respond to sound through movement of structures projecting from the cell, called stereocilia, which eventually leads to release of neurotransmitter from the base of the cell and activation of neurons which send electrical impulses to the central nervous system. The signaling mechanism in the hair cell is poorly understood, but likely involves a complex set of molecular pathways designed not only to convey the signal but to protect the cell from damage caused by over-stimulation.

*Advance:* Using a high performance video imaging system and electron microscopy analysis, scientists have identified an important new feature of hair cells. They showed that there is a localized pool of calcium ions that is released within the cell body in addition to those previously described at the base of the cell. In the present study, focal applications of ATP to the hair bundle of outer hair cells resulted in a two-component increase in intracellular calcium. After the initial entry of calcium through the apical membrane, a second and larger surge of calcium occurred at the base of the cell. Electron microscopy imaging of this release site showed that it coincides with the localization of a unique system of intracellular membranes and mitochondria known as Hensen's body. At this site, release of calcium ions could affect critical structures within the hair bundle.

*Implications:* Studies which investigate the cell biology of hair cells will help determine the mechanisms by which genetic abnormalities that alter the structure of key proteins cause deafness.

Mammano F, Frolenkov GI, Lagostena L, Belyantseva, IA, Kurc M, Dodane V, Colavita A and Kachar B: ATP-induced Ca<sup>2+</sup> release in cochlear outer hair cells: localization of an inositol triphosphate-gated Ca<sup>2+</sup> store to the base of the sensory hair bundle. *J Neurosci* 19: 6918-6929, 1999.

## Identification of Molecular Mechanisms of Pathogenesis of Otitis Media

*Background:* Middle ear infection, or otitis media, is one of the most significant health problems for children in the United States costing approximately 4 to 5 billion dollars annually. It is the most common reason for a sick child to be treated by a physician. NIH-supported investigators continue to focus on the identification of specific molecular mechanisms by which bacterial and viral infections cause otitis media.

*Advance:* The results of two recent studies combine to identify respiratory syncytial virus (RSV) as the principal virus invading the middle ear during acute otitis media and demonstrate significantly enhanced attachment of nontypable *Haemophilus influenzae*, a primary cause of otitis media, when respiratory epithelial cells are infected with RSV. Investigators have shown that attachment of the bacterium to host cells is a crucial step for bacterial induction of otitis media. Importantly, the second study also identifies the specific factors that mediate the RSV-induced attachment of nontypable *Haemophilus influenzae* to respiratory epithelium.

*Implications:* The identification of the steps that lead to otitis media is critical for the development of targeted clinical interventions such as vaccines and other strategies designed to block the infectious process. The successful translation of information obtained through such studies into clinical interventions could significantly reduce the incidence of acute otitis media in children. [secondary B treatment]

Heikkinen T et al: Prevalence of various respiratory viruses in the middle ear during acute otitis media. The New England Journal of Medicine 340(4): 260-264, 1999.

Jiang Z et al: Fimbria-mediated enhanced attachment of nontypeable *Haemophilus influenzae* to respiratory syncytial virus-infected respiratory epithelial cells, Infection and Immunity 67(1): 187-192, 1999.

## **The Vestibular System Influences Cardiovascular Control in Humans**

*Background:* A key role of the autonomic nervous system is to maintain homeostasis, a stable internal milieu, in an organism. A challenge to homeostasis occurs when someone moves or changes their body posture. When a human stands rapidly from a sitting position, the body must detect the disturbance in homeostasis and quickly trigger compensatory responses to avoid blood pooling in the lower body that results in orthostatic hypotension, a fall in blood pressure upon standing associated with dizziness/lightheadedness, syncope (fainting) and blurred vision. While several classes of cardiovascular receptors detect disturbances in homeostasis, homeostatic control during body motion would be optimized if compensatory responses could be triggered by the autonomic centers even before the internal body environment is perturbed. One mechanism for accomplishing this homeostasis would be through the actions of the vestibular system, which senses head position relative to gravity and head motion and relays this information to the brainstem.

*Advance:* Studies in animals show that the vestibular system contributes to cardiovascular control. The signals that trigger vestibulo-cardiovascular responses originate from the vestibular otolithic organs. The neural circuitry linking the vestibular centers and the cardiovascular regulatory centers in the brainstem that mediate these responses have been delineated by investigators. However, definitive evidence that vestibulo-cardiovascular responses occur in humans has heretofore not been provided. A research team has now demonstrated that linear acceleration, a stimulus activating the otolithic organs, elicits rapid changes in the heart rate and blood pressure of human subjects. The cardiovascular responses in patients with marked bilateral loss of vestibular function were much smaller than those of normal subjects.

*Implications:* This finding supports the hypothesis that the vestibular system contributes to maintaining the blood pressure of humans during movement and changes in body posture. The physiologic and clinical significance of vestibulo-cardiovascular responses is currently under active investigation.

Yates BJ et al: Cardiovascular responses elicited by linear acceleration in humans. Experimental Brain Research 125: 476-484, 1999.

## **Taste Receptors**

*Background:* Taste represents a major form of sensory input in the animal kingdom: loss of taste sensation is particularly common among elderly individuals, which can contribute to poor nutrition and loss of a desire to eat. Despite its obvious interest and importance, there is much still to be learned about the cellular and molecular mechanisms critical for taste perception. The molecular pathway resulting in perception of taste is initiated when a sweet, bitter, salty, or sour substance binds to specific taste receptors found on the outer surface of taste cells on the tongue.

*Advance:* In a collaborative effort joining molecular biologists supported by two NIH institutes and the Howard Hughes Medical Institute, the first putative sweet and bitter taste receptors have been cloned and characterized. As predicted from previous research findings, these receptors are members of the superfamily of receptors that signal by interacting with G-proteins, and are selectively expressed in a non-overlapping subset of taste receptor cells on the tongue, along the outer surface of these cells exposed to sweet and bitter substances.

*Implications:* These receptors represent the first step in dissecting the molecular pathway activated by sweet and bitter substances, and will guide future research studies to determine additional molecules in this important, but poorly understood, pathway.

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJP and Zuker CS: Putative mammalian taste receptors: A class of taste-specific GPCRs with distinct topographic selectivity. Cell 96: 541-551, 1999.

## **Deciphering the Code for Odors**

*Background:* The human sense of smell may be the most complex of the senses and it presents some of the most puzzling phenomena to scientists. Humans are able to distinguish many thousands of distinct odors and yet the nose contains a relatively small number of odor receptors to accomplish this impressive task. Even more perplexing are human studies demonstrating that small, subtle changes in the structure of an odor cause dramatic shifts in the perceived odor quality. For example, a small change can cause a pleasant, floral compound to be perceived as rancid and sweaty. Similarly, changes in the concentration of an odor can cause profound shifts in how it is perceived.

*Advance:* Findings reported this year by an investigator offer the first clues to how mammals distinguish different odors and these findings provide a potential explanation for many puzzling phenomena. Molecular approaches were used to identify the receptors activated by different odors. The results showed that each odor activates many different receptors and that receptors are not dedicated to specific odors. Instead, odor identity appears to be encoded by the unique combination of receptors responding to an odor. This information is deciphered by neurons in the brain and results in odor discrimination and perception.

*Implications:* The identification of the coding scheme for odors provides a critical advance in our understanding of the molecular basis of olfactory discrimination.

Malnic B, Hirono J, Takaaki S and Buck LB: Combinatorial receptor codes for odors. Cell 96: 713-723, 1999.

## **Pheromone Pathways of the Brain**

*Background:* The human sense of smell can evoke both conscious perceptions of odors as well as strong emotional and physiological responses to odors. Scientists have known for some time that two separate and functionally distinct sensory organs in the nose mediate responses to odors. Most odors activate neurons in the main olfactory system which sends signals to higher cortical regions responsible for conscious perception. The second system, called the vomeronasal system, is specialized for detecting pheromones, types of odors that are important for social interactions and perpetuation of the species. Signals from the vomeronasal system are transmitted to more primal brain regions, such as the amygdala and hypothalamus that are associated with emotional and innate patterns of responding. Last year, scientists supported by the NIH reported the discovery of a novel family of receptors expressed exclusively in the vomeronasal system that are unrelated to the receptors expressed in the main olfactory system. The existence of unique and unrelated families of receptors in the vomeronasal and the main olfactory systems underscores the difference in the types of odors detected and the separate functional roles of these two sensory systems.

*Advance:* This year, two NIH-funded laboratories have provided our first glimpse of how sensory information in the vomeronasal system is encoded in the brain. Scientists used gene-targeting methods to visualize how axons from neurons in the vomeronasal organ project to their brain targets. The picture that emerges indicates a level of complexity that differs dramatically from the sensory map of the main olfactory system. Axons of vomeronasal neurons project to multiple brain targets in a pattern that is highly variable from one animal to the next. These features contrast sharply with the main olfactory system in which axons project in a highly stereotyped pattern to a small number of discrete brain targets.

*Implications:* Extensive research efforts will be needed to achieve a complete understanding of these results. What is already clear is that the two sensory systems of the nose recognize different arrays of environmental odors and that each system uses different organizational principles to process sensory information and generate an internal representation of odors in the external world.

Rodriguez I, Feinstein P and Mombaerts P: Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. Cell 97: 199-208, 1999.

Belluscio L, Koentges G, Axel R and Dulac C: A map of pheromone receptor activation in the mammalian brain. Cell 97: 209-220, 1999.

### **Protein Has Potential to Treat Brain Damage**

*Background:* Traditionally, it was believed that the number of brain cells was established at birth and unresponsive to signals outside the cell later in life, leaving little hope for efforts to replace old or damaged brain cells. However, the recent identification of neural precursor cells and persistent cell development in the mature brain raises the possibility that the number of neurons in an individual's brain is actively maintained throughout life, rather than being diminished over time.

*Advance:* Scientists have found that a protein in the body, basic fibroblast growth factor (bFGF), regulates nerve cell growth in the brain of newborn rats by crossing the blood-brain barrier to stimulate nerve cell division. These researchers also discovered that the effects of bFGF were not restricted to the perinatal period, but also stimulated brain cell growth in *older* animals, indicating that cells continue to be responsive to bFGF later in life. In adult animals, peripheral bFGF increased cell division threefold in the forebrain and olfactory tract, indicating that bFGF regulates ongoing generation of nerve cells by a unique, internal secreting pathway, potentially providing new approaches for treating damaged brain cells during development and into adulthood. Researchers also found that the peripheral injection of small doses of bFGF also increased the proportion of early nerve cells and stimulated the growth of new nerve cells in the neonatal rat brain. Moreover, the fact that bFGF enters the brain to stimulate cell division suggests an ongoing communication between nerves and their target tissues.

*Implications:* Results indicate that bFGF rapidly crosses the blood-brain barrier throughout life, regulating the division of early nerve cells during development as well as in adulthood. The existence of a biological pathway transporting growth factors to the nervous system has potential implications for developing treatments for brain damage associated with neurodegenerative conditions, such as Alzheimer's, in addition to congenital conditions and acquired brain disease. [secondary B treatment]

Wagner JP, Black IB, and DiCicco-Bloom E: Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. J Neuroscience 19: 6006-16, 1999.

## Preventing Early Miscarriage

**Background:** Implantation is the process whereby a developing embryo attaches to the uterine wall. In order to attach, the embryo must invade the uterine lining via the layer of cells that subsequently develop into the placenta, and exchange important signals with maternal tissues. We still have much to learn about the implantation process.

**Advance:** Scientists investigated how the hormones progesterone and estrogen induce implantation of the mouse embryo. First, they studied estradiol (E<sub>2</sub>), an estrogen that is necessary to prepare the uterus for implantation. Then, they studied the hormone catecholestron, a metabolic product of estrogen, which is important for the ability of the embryo to implant in the uterine lining. They showed that a mouse uterus which is receptive to embryonic implantation synthesizes catecholestron. Thus, estrogen acts in a dual fashion essential to promote implantation -- with E<sub>2</sub> preparing the progesterone-primed uterus for the acceptance of the embryo, and its metabolite, catecholestron, mediating the embryo's ability to implant. These scientists also studied how the uterus responds to progesterone. *Hoxa-10* is a gene expressed in the developing mouse's genitourinary tract and its absence causes infertility in male and female mice. They found that during implantation the gene's product, Hoxa-10, directly influences progesterone activity, specifically affecting progesterone regulation of the E<sub>2</sub> receptor of prostaglandin (a mediator of various physiologic processes). Mice without the *Hoxa-10* gene have poor implantation, suggesting that a *Hoxa-10* mutation causes specific uterine defects that can lead to problems in implantation. Earlier, the researchers found that mice deficient in an enzyme essential for synthesizing prostaglandin are infertile. They further demonstrated the essential role of the prostaglandin PGI<sub>2</sub> in implantation and uterine lining development during gestation.

**Implications:** It is critical to gain detailed knowledge of implantation, since it is thought that a large percentage of early spontaneous abortions (miscarriages) in humans occur during the implantation process or shortly thereafter. Clinically recognized miscarriages occur in about 15-20% of known human pregnancies, although the true embryonic loss is estimated to be much higher. The risk of miscarriage or spontaneous abortion is increased with age. For women over age 40 the risk is 25-30%, as opposed to 15-20% at age 25 or less. For men at age 45 the risk of a miscarriage in his mate is double that of a man age 25 (23% vs. 12%, respectively). It is therefore, extremely important to understand the process of implantation to help prevent pregnancy loss. [secondary B prevention]

Lim H, Gupta RA, Ma W, Paria BC, Moller DE, Morrow JD, DuBois RN, Trzaskos JM, and Dey SK: Cyclooxygenase-2 derived prostacyclin mediates embryo implantation in the mouse via PPAR. Genes and Development 13: 1561-1574, 1999.

Lim H, Ma L, Ma W, Maas RL and, Dey SK: *Hoxa-10* regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse. Mol. Endo. 13: 1005-1017, 1999.

Paria BC, Lim H, Wang X-N, Liehr J, Das SK, and Dey, SK: Coordination of differential effects of primary estrogen and catecholestron on two distinct targets mediates embryo implantation in the mouse. Endo. 139: 5235-5246, 1998.

## Improving Treatment for Polycystic Ovary Syndrome

**Background:** Polycystic ovary syndrome (PCOS) is a condition of excess androgen (a hormone that leads to masculinization). PCOS affects about 5-10% of reproductive-age women. Classical symptoms include excess hair growth, ovulatory dysfunction with menstrual irregularity and infertility, obesity and insulin resistance. Women with this syndrome who do get pregnant may be at increased risk for gestational diabetes. PCOS metabolic abnormalities may also raise the risk of cardiovascular disease. Current treatments are directed at the symptoms, not the underlying disease process. Recently, several advances have converged to shed light on PCOS, to improve its diagnosis and treatment.

**Advance:** Scientists have successfully generated a transgenic mouse model that mimics many features of PCOS. When these mice were induced to ovulate and mated, pregnancy was either never established because embryos could not implant in the uteri, or it failed mid-gestation. Their ovulated eggs, however, produced healthy babies when transferred to normal mouse uteri.

In human studies, other scientists analyzed genes to identify those playing a role in causing PCOS and found that the strongest candidate was the follistatin gene. Since an increase in follistatin or its activity could arrest development of ovarian follicles, increase ovarian androgen production and impair insulin release (all characteristic of PCOS), these findings merit follow-up. A potential new treatment of this syndrome has also been reported. Women with PCOS have insulin resistance and excessive insulin in their blood. Using knowledge of how insulin acts in glucose metabolism, the investigators reasoned that women with PCOS may have deficiencies in this physiological process. They administered *D-chiro*-inositol (a natural substance that promotes the proper breakdown and use of glucose by regulating insulin action) or placebo to 44 women with PCOS. Nineteen of the 22 women on the drug ovulated as compared to 6 of the 22 in the placebo group. The drug treatment group also showed improvement in endocrine and metabolic abnormalities.

**Implications:** The first two studies highlight research that may improve diagnosis and treatment of PCOS. The transgenic mouse model is very useful for understanding PCOS, while the genetic linkage study shows how a systematic screen of candidate genes can provide clues for the causes of a complex disease, and can identify genes for further study. The *D-chiro*-inositol study demonstrates how basic research can be applied to clinical practice. Together, these studies show how management of PCOS and its associated infertility is moving toward treating the underlying disease process. [secondary B treatment]

Mann RJ, Keri RA, and Nilson JH: Transgenic mice with chronically elevated luteinizing hormone are infertile due to anovulation, defects in uterine receptivity, and midgestation pregnancy failure. Endo. 140: 2592-2601, 1999.

Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss III JF, Spielman RS, and Dunaif A: Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Nat Acad Sci 96: 8573-8578, 1999.

Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, and Allan G: Ovulatory and metabolic effects of *D-chiro*-inositol in the polycystic ovary syndrome. N Eng J Med 340: 1314-20, 1999.

### **Islet-Specific Transcription Factors and Development of the Endocrine Pancreas**

*Background:* The pancreas serves two major roles: its exocrine role is involved with secreting digestive enzymes into the intestines, and its endocrine role is involved with secreting hormones to regulate glucose levels in the body. Diabetes mellitus occurs when the endocrine pancreas malfunctions and the body fails to metabolize carbohydrates properly. The endocrine pancreas is composed of four types of islet cells (alpha, beta, gamma and PP cells), which secrete glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. Each of these secreted proteins play a key role in regulating food intake and metabolizing key nutrients. Since all four types of endocrine islet cells arise from a population of common cells, scientists hypothesize that a unique set of transcription factors (genes that direct protein assembly) determine the final outcome for these cells.

*Advance:* Major progress has been achieved toward identifying genes important for islet cell development. One gene, *BETA2*, was recently discovered and found to be important in regulating insulin. Mutation in this gene disrupts proper islet cell formation. Animals with this mutation develop severe diabetes and die shortly after birth. This finding shows the importance of the *BETA2* gene in the development of pancreatic islet cells. In addition, these researchers have recently isolated a protein from pancreatic cells, *ngn3*, which appears to regulate the activity of *BETA2* in the early stages of pancreas development.

*Implications:* Knowledge of the relationship of pancreatic genes and their regulators will lead to a better understanding of the molecular mechanisms controlling endocrine pancreas formation and will contribute to the development of new therapeutic approaches for diabetes.

Huang H-P and Tsai M-J: Transcription factors involved in pancreatic islet development. *J Biomed Sci* 1999; In Press.

## Effects of Estrogen on the Brain After Menopause

*Background:* Menopause is perhaps the single most influential biological or health-related event for most middle-aged women, and it is estimated that the average woman will spend at least half of her adult life with decreased levels of estrogen. Decisions concerning hormone replacement therapy represent major concerns for postmenopausal women. The declining estrogen levels that define menopause affect a range of organ systems including the reproductive system, the cardiovascular system, and the skeletal system. There is also evidence that lack of estrogen affects basic cognitive functions, such as memory. Several previous studies have examined the influence of estrogen on cognitive function, but results have been inconsistent. Recent advances in technology now permit a noninvasive, highly sensitive way of measuring brain function as individuals perform memory and other cognitive tasks.

*Advance:* In a groundbreaking new study, researchers used sophisticated brain imaging technology to show that estrogen alters brain activation patterns in postmenopausal women as they perform memory tasks. Researchers used a technology called functional magnetic resonance imaging (fMRI), which produces computer-generated images of blood flow in the brain during such tasks as thinking, reading or remembering. Researchers in the study used these images to compare brain activation patterns in postmenopausal women when they were taking estrogen to when they were taking a placebo. The women were then asked to perform simple tasks designed to test their ability to store verbal information and hold it in memory for brief periods of time (e.g., remembering a telephone number). While women on estrogen received roughly the same numerical scores on the tests as women on placebo, researchers found that estrogen changed the brain activation patterns of the postmenopausal women and that the changes resembled brain activation patterns typically seen in younger people.

*Implications:* These findings show that it is possible to alter brain organization in older individuals, indicating that the memory systems of mature women are responsive to external stimuli rather than being fixed or immutable. In addition, these results suggest that the use of functional imaging, coupled with protocols examining proper dosing regimens for estrogen, may yield important new information concerning the effects of estrogen on cognitive function in postmenopausal women. This information may offer better options for preventing and treating cognitive deficits in this population. [secondary B prevention]

Shaywitz SE, Shaywitz BA, Pugh KR, Fulbright RK, Skudlarski P, Mencl WE, Constable RT, Naftolin F, Palter SF, Marchione KE, Katz L, Shankweiler DP, Fletcher JM, Lacadie C, Keltz M, and Gore JC: Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *JAMA* 281: 1197-1202, 1999.

### **Identifying a Risk Factor for a Common Birth Defect**

*Background:* It is well known that folic acid can prevent some birth defects (such as spina bifida) when taken before conception. One way this occurs is by overcoming an abnormal enzyme caused by a gene defect. This abnormal enzyme, called thermolabile methylenetetrahydrofolate reductase (MTHFR), is responsible for reduced availability of folate for many important chemical reactions. Ingesting sufficient folic acid can overcome this problem.

Many studies have suggested that cleft lip and cleft palate can also be prevented by folic acid; others have found no protective effect whatsoever. This study addressed this issue by looking for the abnormal thermolabile MTHFR in subjects with clefts and normal control subjects. The reasoning behind this approach was that if subjects with clefts were more likely to have the abnormal enzyme, it would provide a possible mechanism by which folic acid could prevent clefts.

*Advance:* Patients with cleft palates were significantly more likely to have the abnormal thermolabile MTHFR genes than normal control subjects. This suggests a potential mechanism by which folic acid could be related to clefts, since taking folic acid would overcome the block created by MTHFR and allow the embryo to develop normally. As in all gene-enzyme studies, this requires confirmation in other populations.

*Implications:* These findings indicate that the same gene that is a risk factor for neural tube defects is associated with cleft palate. This provides important new insight into the mechanism of this defect and provides potential avenues for prevention. [secondary B prevention]

Mills JL, Kirke PN, Molloy AM, Burke H, Conley MR, Lee YJ, Mayne PD, Weir DG, and Scott JM: Methylenetetrahydrofolate reductase thermolabile variant and oral clefts. Amer J Med Gen, 86: 71-74, 1999.

### **NK Cells and Resistance to Cancer Metastasis**

*Background:* Cancer among children is second only to accidents as the leading cause of death. Very young children, aged 0 to 4 years, have a higher prevalence of cancer than children in the 5 to 9 or 10 to 14 year range. There are few explanations why so many very young children have cancer.

Animal studies have linked resistance against cancer to the presence of natural killer cells, a type of blood cell. In this study the natural killer cells were removed from both young and mature groups of rats in order to examine the role of natural killer cells in resistance against cancer in young animals. Both groups were injected with a known number of cancer cells. Although blood levels of corticosteroids vary with age and with gender, their role in killer cell activity has not been explored.

*Advance:* The study supported the idea that the susceptibility of very young animals to metastasis of cancer cells is related to reduced killer cell activity. This study did not find evidence that corticosteroid levels affected either natural killer cell activity or how many injected cancer cells were retained.

*Implications:* Although natural killer cells are only one mechanism used by immune systems, the resistance of young animals to cancer seems related to natural killer cell activity. This finding suggests the possibility that drugs or other substances which increase natural killer cell number and/or activity hold promise for treating, or even preventing, cancer among the very young. [secondary B treatment]

Page GG, Ben-Eliyahu, S: A role for NK cells in greater susceptibility of young rats to metastatic formation. Developmental and Comparative Immunology 23: 87-96, 1999.

## **Gender Differences in Heart Muscle Function**

*Background:* Gender differences have been reported in both animal and human heart muscle function. Between men and women, there are a range of heart function differences but scientists can't explain how the differences occur. These differences include exercise-associated increase in ejection fractions of men (a measure of blood volume per heart beat), and in women, longer QT intervals (distance between specific points on electrocardiogram tracings). If the bases of these differences were discovered, then treatment could be developed more specifically for men and for women, in order to achieve the best outcomes in treatment of heart problems.

The current study utilized an animal model to describe gender differences in the contraction of heart muscle. Two possible mechanisms for gender differences in heart muscle function were explored: a measure of calcium metabolism and the response to beta adrenergic stimulation. Results indicate a greater response to beta and alpha adrenergic stimulation in male rat heart muscle compared to female.

*Advance:* The study suggests further gender differences in heart muscle contraction within an animal model. The study also provided additional information about possible explanatory mechanisms underlying these differences: calcium metabolism and response to beta adrenergic stimulation. The specific parameters investigated are similar in mechanism to those used by several cardiac medications in current use for human heart disease.

*Implications:* This study adds to the growing volume of studies describing gender differences in health and illness. Several medications in current use to treat human heart disease employ mechanisms similar to the mechanisms examined in this study. This implies that different medications and/or doses of medications might be found to achieve the best results in women compared to men. [secondary B treatment]

Schwartz D, Vizgirda V, Solaro, RJ, Piano, MR, Ryjewski C.: Sexual dimorphism in rat left atrial function and response to adrenergic function. Molecular and Cellular Biochemistry 200: 143-153, 1999 (in press).

## **Complement System May Be Useful Target for the Treatment of Sepsis**

*Background:* Sepsis is a catastrophic inflammatory condition caused by infection that results in multi-organ system failure. Although treated with antibiotics in intensive care units, more than 40 percent of sepsis patients still die from the condition. Finding a more effective treatment for these patients has long been a goal of researchers.

*Advance:* Investigators recently produced surprising results that may explain the cause of some of the impaired ability of the body to fight off bacterial infections that is a common feature of sepsis. The complement system, a cascade of blood proteins that leads to the recognition and destruction of foreign pathogens by white blood cells, has an essential role in clearing bacteria from the body. But investigators showed that blocking one component in the complement cascade (by pretreatment with an antibody) actually reduced the amount of bacteria in blood and tissues (and dramatically lowered mortality) in experimental animals. In addition, white blood cells were better able to defend against the foreign pathogens when the complement was blocked. These studies provide new, unexpected findings about what may occur during sepsis.

*Implications:* These results suggest that the complement system may be a useful target for the treatment of sepsis. One disease area where an effective treatment for sepsis could save many lives each year, and many dollars in treatment costs, is acute respiratory distress syndrome (ARDS), a serious inflammatory lung condition in which sepsis has a major role. Presently, patients with ARDS spend many days, and sometimes weeks, in intensive care units. Still, as many as 60,000 die each year. Greater understanding about the role of the complement system in sepsis could lead to development of more effective, less costly treatments for diseases like ARDS. [secondary B treatment]

Czermak, BJ, Sarma V, Pierson CL, Warner RL, Huber-Lang M, Bless NM, Schmal H, Friedl HP, Ward PA: Protective effects of C5a blockade in sepsis. Nature Medicine 5:788-792, 1999.

### **Substance Abuse Treatment Can Be Cost-effective**

*Background:* Recent health insurance reform has excluded drug abuse from Federal legislation that mandates a limited parity for mental health and medical care. Most employer-sponsored health plans limit the type or quantity of substance abuse services available to members. One reason substance abuse is excluded from many health plans is uncertainty about costs and fear about cost inflating mental health care utilization. With the growth of managed care delivery systems have changed dramatically. Between 1992 and 1997, managed behavioral health care companies doubled their enrollment from 78 to 150 million members. Generally, cost estimates have not taken into account this fundamental change in the management of substance abuse benefits.

*Advance:* To calculate the impact that substance abuse parity has on health care costs, researchers conducted a study of substance abuse treatment utilization and costs in a number of plans managed by a large managed behavioral health care company. In this analysis, researchers compared no limit plans to plans with annual limits on substance abuse treatment. The researchers found that changing even stringent limits on annual substance abuse benefits had only a small absolute effect on overall insurance costs under managed care, even though a large percentage of substance abuse patients were affected. For example, removing an annual limit of \$10,000 per year on substance abuse treatment was estimated to increase insurance payments about by \$0.06 per member per year. Removing an annual limit of \$1,000 was estimated to increase payments about \$3.40.

*Implications:* There is a common belief that unlimited substance abuse benefits would dramatically increase health care costs. Because of these concerns, some employers have started to separate mental health and substance abuse benefits. This study, however, shows that these concerns are unfounded and that parity benefits under managed care would be affordable.  
[secondary B treatment]

Sturm RS, Zhang W, and Schoenbaum M: How expensive are unlimited substance abuse benefits under managed care? Journal of Behavioral Health Services and Research, 26(2), 203-210; 1999.

## Chemical Identified That Can Block Brain Damage Caused By Methamphetamine

*Background:* Methamphetamine has been shown to destroy brain cells. Scientists have now identified a chemical that may help combat this damage. This chemical, known, as [D-Ala<sup>2</sup>,D-leu<sup>5</sup>]enkephalin (DADLE) is similar to a chemical, enkephalin, normally produced by the brain. Previous research has found that DADLE would significantly increase the survival time and improve the condition of organs, such as lungs and hearts, that are awaiting transplantation. Based on these results, scientists hypothesized that DADLE has tissue protective properties at least in peripheral organs. In the present study, scientists investigated the possibility that DADLE may also have protective properties in the brain.

*Advance:* The psychostimulant methamphetamine is a drug of abuse that can cause long-term damage to brain neuron cells that contain the neurotransmitter dopamine. This damage, or neurotoxicity, is primarily found at the ends of nerve cell processes, called nerve terminals. Researchers have recently shown in animals that DADLE can not only block the damage to dopamine containing nerve terminals due to subsequent use of methamphetamine, but it can also reverse existing damage caused by the prior use of methamphetamine. Additionally, experiments with cells grown in culture indicate that the protective actions of DADLE in promoting cell survival are long-acting and potent.

*Implications:* These exciting results identify for the first time a potential means of preventing and even reversing nerve cell damage caused by drug use. This has important implications for treating drug addicts and the sequelae of problems that result from drug use. Additionally, DADLE's neuronal protective properties may help prevent or reverse the brain damage caused by brain illnesses such as stroke, Parkinson's Disease, or aging. Ongoing research may clarify DADLE's use as a therapeutic agent to combat many brain diseases with an ultimate benefit to countless individuals. [secondary B prevention and treatment]

Tsao, L-I, B Ladenheim, A Andrews, CC Chiueh, JL Cadet and T-P Su (1998): *Delta* opioid peptide [D-Ala<sup>2</sup>,D-Leu<sup>5</sup>]Enkephalin blocks the long-term loss of dopamine transporter induced by multiple administrations of methamphetamine: Involvement of opioid receptors and reactive oxygen species. *J. Pharmacol. Exp. Ther.* 287:322-331.

### **A Chemical Produced in the Brain May Offer New Insights into Tourette's Syndrome and Parkinson's Disease**

*Background:* Researchers have known for many years that the major psychoactive ingredient in marijuana is tetrahydrocannabinol (THC). Although it was known that THC interacts with specific sites, known as receptors, in the central nervous system it wasn't until the early 1990's that researchers isolated a chemical produced by the brain itself that interacted with these THC receptors. This chemical, named anandamide, was found to be involved in a variety of biological activities, such as analgesia, sedation, memory and cognition.

*Advance:* In a recent study using rats, researchers have discovered that anandamide also helps regulate movement. Movement is a very complex function that involves many brain areas and different neurotransmitters, including dopamine. In this study the researchers determined that in normal rats dopamine caused the release of anandamide from nerve cells. The released anandamide then reduced the amount of dopamine released by other neurons which resulted in a reduction in movement. This system of checks and balances appears to be integral to controlling and fine-tuning muscle movement. In this study researchers also found that if they prevented anandamide from affecting the levels of dopamine, movements were enhanced.

*Implications:* This finding may have implications for disorders such as schizophrenia, Tourette's Syndrome and Parkinson's Disease, all of which appear to involve dysregulated dopamine neurotransmission. These results suggest that anandamide may hold clues to better understanding these disorders which, in turn may lead to effective treatments. Furthermore, since all drugs of abuse produce their effects through the dopamine system, an increased understanding of how anandamide regulates and interacts with dopamine may open new avenues for treatments for drug addiction. [secondary B treatment]

Giuffrida A, Parsons LH, Kerr TM, deFonseca FR, Navaro M, and & Piomelli D: Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nature Neuroscience, 2(4): 358-363, 1999.

## **Chronic Marijuana Smokers May Undergo Withdrawal When They Quit**

*Background:* Motivations for drug abuse have been traditionally attributed to either relief from unpleasant side effects of drug withdrawal, or a desire to experience the positive, pleasurable effects of the drug experience. For opiate drugs like morphine and heroin, a profound physical withdrawal syndrome is experienced upon cessation of drug taking. For psychostimulants like amphetamine or cocaine, while a physiological abstinence syndrome is not apparent upon abstinence, abusers report an unpleasant psychological state. Until recently, it was unknown which of these motivating variables (drug reinforcement or relief from withdrawal) contributed to abuse of marijuana.

*Advance:* Two scientific studies published in 1999 suggest that cessation of marijuana smoking may also produce an unpleasant withdrawal state. In one study, researchers provided frequent marijuana smokers with cigarettes treated with the active ingredient in marijuana  $\Delta^9$ -tetrahydrocannabinol (THC). After the volunteers smoked these cigarettes in a residential laboratory environment for four days, they reported feelings of irritability and stomach pain when the cigarettes were no longer available. They also reported feeling less social and talkative during this abstinence period. In another study, researchers found that long-term marijuana users brought into the hospital and denied access to the drug displayed more aggressive behavior on a computer test of aggression at three to seven days after discontinuing marijuana use.

*Implications:* These scientific observations have profound implications for how we think about marijuana smoking. They clearly demonstrate that long-term marijuana users can experience withdrawal symptoms and that effective treatments to alleviate these symptoms are needed. They also suggest that continued use of marijuana may be an effort to alleviate an unpleasant abstinence syndrome. Lastly, these findings suggest that there is a complex set of physical and subjective variables directing the continued abuse of smoked marijuana, similar to what has been found for other drugs of abuse. [secondary treatment]

Haney M, Ward A, Comer SD, Foltin RW and Fischman MW: Abstinence symptoms following smoked marijuana in humans. Psychopharmacology 141: 395-404, 1999.

Kouri EM, Pope HG Jr and Lukas SE: Changes in aggressive behavior during withdrawal from long-term marijuana use. Psychopharmacology 143: 302-308.

### **Dopamine: More than Just the Pleasure Molecule**

*Background:* Dopamine's role in addiction may be not quite as simple as previously thought, that is as the brain's "feel-good" chemical. In the early 1970's researchers discovered that cocaine, heroin and other addictive drugs, as well as natural rewards such as food and water, would increase the levels of dopamine in certain brain regions. Other studies found that animals would readily learn to press a lever to deliver an electrical stimulus to specific brain regions—those containing dopamine. These studies led many researchers to dub dopamine as the "pleasure chemical," believing that excess dopamine in certain brain regions was perceived by the organism as a pleasurable sensation. A new study is now showing that dopamine's role is more complex than was previously thought.

*Advance:* Using state-of-the art technology, researchers can now measure the role of dopamine in drug reward with a finer temporal resolution. Voltametry, which uses a specially treated carbon fiber tip to detect minute amounts of neurochemicals, represents a major advance over microdialysis in the measurement of neurotransmitters in the brain. New findings in animals suggest that in addition to promoting feelings of well-being, dopamine also seems to have an alerting function, helping animals to notice what is new in their environment. It may act to make new experiences more pleasurable, that is it may serve as a predictor of a reward or pleasurable sensation, but once the pleasurable task is learned dopamine's role is diminished. Because dopamine's role may be more varied and complex than previously thought, drug treatment approaches need to be designed that focus on more than just blocking dopamine.

*Implications:* As researchers learn more about the neurobiological processes that underlie addiction, more efficacious treatments, both pharmacological and behavioral can be designed. While the dopamine system is still important, it appears that its role may be particularly critical in the initial response to drugs of abuse and that other neural systems may become more critical as an individual progresses from initial drug use to addiction. Identification of the role that other brain systems play in addiction will be important in our efforts to treat addiction and relapse. [secondary B treatment]

Garris, PA, Kilpatrick, M, Bunin, MA, Michael, D, Walker, QD and Wightman, RM. Dissociation of dopamine release in the nucleus accumbens from intracranial self-stimulation. Nature; 398(4):67-69, 1999.

## Health Effects of Cigar Smoking

*Background:* The sale of cigars in the United States has been increasing since 1993, reversing a 20-year decline in the rate of cigar consumption. The resurgence of cigar smoking may be attributed, in part, to the perception that cigars are safer than cigarettes. However, few data are available on the health effects associated specifically with cigar use, especially with regard to cardiovascular disease risk.

*Advance:* In this study, 17,774 men ages 30 to 85 who were enrolled in the Kaiser Permanente health plan and were not cigarette smokers were followed through the end of 1995. Researchers found that cigar smokers, as compared with nonsmokers, were at increased risk for cancers of the lung and the upper aerodigestive tract (including the top of the throat, the nose, the larynx, and the esophagus), cardiovascular disease, and chronic obstructive pulmonary disease, independently of other risk factors for these diseases. Risks were greater among those who smoked five or more cigars a day compared to those who smoked fewer. There appeared to be a synergistic relationship between alcohol consumption and cigar use with respect to risk of cancers of the upper aerodigestive tract and parts of the throat.

*Implications:* Far from being a safe form of recreation, cigars pose significant health risks to people who smoke them. Their increasing popularity is rapidly emerging as a troubling public health issue. [secondary B prevention]

Iribarren C, Tekawa IS, Sidney S, and Friedman GD: Effect of cigar smoking on the risk of cardiovascular disease, chronic obstructive pulmonary disease, and cancer in men. New England Journal of Medicine 340: 1773-1780, 1999.

## Creating Human Cancer Cells

*Background:* After years of study, we know that cancer arises through a multistep process involving the accumulation of genetic alterations & abnormalities that enable a cell to override the normal mechanisms that control cellular proliferation and divide on its own schedule. Despite this important knowledge, scientists have struggled for more than 15 years to create human cancer cells in a cell culture dish. Some scientists have used chemical or physical agents to transform normal cells into cancer with limited success. And, although a team of researchers showed more than a decade ago that changes in two specific genes promoting cell growth caused rodent cells to become cancerous, similar experiments using human cells failed. Until now, no one has been able to identify the minimum number of defined genetic events needed to transform a human cell from one that is normal to one that will continue to proliferate indefinitely.

*Advance:* In a striking achievement, a team of NIH-supported scientists recently converted normal human cells to tumor cells in a culture dish by altering the expression of a defined set of genes, changing at least four pathways in the cell. Using two different cell types & done to ensure that the observed effects were not limited to a particular type of cell & the scientists first artificially stimulated the expression of the telomerase enzyme, hTERT, which maintains telomere length (pathway 1). Telomeres, specialized structures that define the ends of chromosomes, become increasingly shorter every time a cell divides; such shortening may serve to limit a cell's lifespan. Stimulating expression of hTERT overcame telomere shortening and allowed the cells to proliferate indefinitely. They then added two familiar oncogenes: an activated *ras* gene found in many human tumors which stimulates cellular growth (pathway 2) and a gene from the simian virus 40 (SV40) retrovirus that encodes for the large T protein. This protein transforms normal cells infected with SV40 by inactivating the cellular pathways controlled by the p53 and retinoblastoma tumor-suppressor proteins (pathways 3 and 4). The delivery of all three elements & hTERT, an activated *ras* oncogene, and the large-T protein & transformed normal human cells grown in cell culture, causing them to behave as cancer cells. Because all three elements were needed to convert the normal cells, the scientists concluded that mutations activating telomerase cooperate with other alterations in oncogenes to transform normal human cells to cancerous ones. In addition, when the transformed cells were injected into mice, the animals developed tumors, confirming that these cells developed in a cell culture system were indeed capable of producing tumors in living animals.

*Implications:* The ability to introduce specific genetic alterations to transform normal cells provides the exciting opportunity to define the biochemical pathways in the cell that must be disrupted in the development of cancer. Such information will open new avenues for exploring the roles of various cellular pathways that become disrupted and for determining the sequence events that must occur as cancer develops. For example, using the approach developed by these investigators, we now can determine whether the *ras* oncogene must be activated before or after the introduction of SV40 for cancer to develop. Such information will be useful for developing treatments that target specific steps in cancer development.

Weitzman JB and Yaniv Y: Rebuilding the Road to Cancer. *Nature* 400: 401-402, 1999. Hahn WC, Counter CM, Lundberg AS, et al.: Creation of human tumour cells with defined genetic elements. *Nature* 400: 464-468, 1999.

## Published Research Using Linked SEER-Medicare Data

*Background:* Cancer registries gather a wide variety of specific information on cancer patients; this information can then be analyzed to identify trends. The Surveillance, Epidemiology, and End Results Program (SEER) is a continuing project of the NIH to collect cancer data on a routine basis from designated population-based cancer registries in various areas of the country. Trends in cancer incidence, mortality and patient survival in the United States are derived from this data bank. The geographic areas covered by the SEER Program's data base represent an estimated 14 percent of the United States population. The SEER-Medicare database links information from the cancer registries with Medicare-eligible persons reported to the SEER registries. The studies discussed here indicate that information linkages between cancer registries and other large sources of data can be important research tools.

*Advance:* In several studies using the linked SEER-Medicare data, researchers found that claims such as those collected by Medicare will capture 75 to 90 percent of cancer cases supported by the cancer registries, completeness varying by the type of cancer, although data from cancer registries remains an extremely important source of information. One study found that using the Medicare claims to determine if a person had a newly diagnosed breast cancer had significant limitations; another found that using the Medicare data offers the potential to enhance the census and evaluation of adjuvant treatments, such as radiation therapy, that are supposed to be recorded by registries, but are sometimes missed as the care is provided in an alternate location.

The SEER-Medicare data were also used to examine the ways patterns of care differ between health maintenance organizations (HMOs) and fee-for-service (FFS) settings. By 1996, approximately 20 percent of the U.S. population, and about 14 percent of Medicare recipients, were enrolled in HMOs, a significant increase since 1980.

In three studies, NIH researchers and grantees compared treatment and outcomes among Medicare recipients in HMOs and FFS settings.

- \$ In the first study, researchers studied the care received by colorectal cancer patients within the geographic areas served by two large, not-for-profit HMOs. They found little difference in treatment and cancer-specific mortality between patients treated in HMOs and patients treated in the FFS setting.
- \$ In the second study, researchers looked at the same geographic area and found that 10-year survival following a diagnosis of non-metastatic prostate cancer diagnosis was similar for men treated in each setting, although there were significant differences in treatment between the FFS and one of the HMOs.
- \$ In the final study, researchers compared differences in stage and treatment for older women with breast cancer. The investigators found that women in HMOs were diagnosed at an earlier stage. The use of lumpectomy for early-stage cancers was similar between HMOs and FFS, although the recommended radiation therapy following lumpectomy was more likely to occur in HMOs. The type of care received by women within HMOs varied widely B some HMOs had high rates of breast-conserving surgery, others had very low.

Given the variation in the type of care among HMOs, comparing HMOs as a group with FFS as a group may have limitations; however, as the number and types of health care plans continue to increase, it will be important to monitor the effects of alternative delivery systems on cancer detection and treatment.

*Implications:* The use of the combined SEER-Medicare data will allow us to conduct important research on treatment delivery and outcomes. The second set of findings reported here underscores the benefit of linking data from cancer registries with large sources of claims, such as the Medicare data, while the first set provides an example of how the combined data can be used. [secondary B treatment]

Cooper GS, Yuan Z, Stange KC, et al.: The sensitivity of Medicare claims data for case ascertainment of six common cancers. Medical Care 5: 436-444, 1999.

Du X, Freeman JL, and Goodwin JS: Information on radiation treatment in patients with breast cancer: The advantages of the linked Medicare and SEER data. Journal of Clinical Epidemiology 52: 463-470, 1999.

Warren JL, Feuer E, Potosky AL, Riley GF, and Lynch CF: Use of Medicare hospital and physician data to assess breast cancer incidence. Medical Care 5: 445-456, 1999.

Riley GF, Potosky AL, Klabunde CN, Warren JL, and Ballard-Barbash R: Stage at diagnosis and treatment patterns among older women with breast cancer. Journal of the American Medical Association 281: 720-726, 1999.

Potosky AL, Merrill RM, Riley GF, et al.: Prostate cancer treatment and ten-year survival among group/staff HMO and fee-for-service Medicare patients. Health Services Research 34: 5240546, 1999.

Merrill RM, Brown ML, Potosky AL, et al.: Survival and treatment for colorectal cancer Medicare patients in two group/staff health maintenance organizations and the fee-for-service setting. Medical Care Research and Review 56: 177-196, 1999.

## Interpretation of Emerging Patterns and Trends in Cancer

*Background:* Appropriate decision making in science and in public health depends on accurate, reliable information about the incidence and impact of disease. The NIH measures the national burden of cancer through incidence, morbidity, mortality, and survival statistics, and evaluates the impact of cancer-related risk factors, health behaviors, and health services on the trends observed.

*Advance:* Cancer incidence rates declined approximately 0.9 percent a year between 1990 and 1996, and cancer death rates fell approximately 0.6 percent a year during the same period, according to a 1999 report of the NIH, the American Cancer Society, the Centers for Disease Prevention and Control, and the National Center for Health Statistics. Their Report to the Nation outlined major cancer trends in the 1990s. The investigators noted that overall, cancer incidence and death rates have declined significantly since 1990. In particular, lung cancer rates for men (although not for women) appear to be on the decline. These trends are encouraging, but the investigators caution that unless recent upward trends in smoking among adolescents are reversed, lung cancer rates may begin to rise again.

NIH scientists have also inaugurated the Cancer Surveillance Series of research articles that address the emerging patterns of cancer in various population groups in the United States, and explore the many elements (e.g., risk factors, screening, diagnosis and treatment) affecting these patterns at the national and regional levels. The series also publicizes the data sources and systems that are available for cancer surveillance research, and provides a forum for wide dissemination of the latest analysis and evaluation of cancer statistics in the United States. The series was inaugurated in June 1999 in the *Journal of the National Cancer Institute*. Some of the findings reported in that issue include:

- \$ In a three-part article, investigators evaluated the incidence and mortality trends from prostate cancer and the impact of prostate-specific antigen (PSA) screening (a common blood test that frequently indicates the presence of prostate cancer), attribution bias, and other factors. They found that since PSA screening was introduced in the 1980s, the incidence of advanced prostate cancer has declined, as has the overall prostate cancer mortality rate, indicating that such screening is enabling earlier detection and *possibly* a sustained decline in the prostate cancer death rate. Investigators further report that some recently diagnosed patients who die of other causes may be mislabeled as having died of prostate cancer. Finally, they note that it is unlikely that the entire decline in prostate cancer mortality can be explained by PSA testing. The investigators note the complexity of the data, and indicate that there is some evidence that PSA screening leads to a decrease in overall prostate cancer deaths, although alternative interpretations are possible.
- \$ There was no substantial change in incidence for the major pediatric cancers, and rates have remained relatively stable since the mid-1980s. The modestly increased incidence reported in certain cancers was confined to the mid-1980s, and probably reflected improvements in diagnosis, or changes in reporting. Mortality rates declined steadily for all major childhood cancers due to dramatic improvements in treatments. However, those rates declined less rapidly for brain and other central nervous system cancers in children.

\$ Geographic patterns of lung cancer have changed considerably since 1950, and have generally coincided with regional trends in cigarette smoking. This finding indicates that public health

measures aimed at smoking prevention and cessation could be highly effective in reducing lung cancer rates.

*Implications:* NIH uses its surveillance activities to identify key trends in cancer B both the good news (significant dips in cancer incidence and mortality) and the bad (an increase in smoking among adolescents). By interpreting these trends, NIH will be able to direct education and cancer prevention efforts where they are most needed. [secondary B prevention]

Hankey BF, Feuer EJ, Clegg LX, et al.: Cancer Surveillance Series: Interpreting trends in prostate cancer B Part I: Evidence of the effects of screening in recent prostate cancer incidence, mortality, and survival rates. Journal of the National Cancer Institute 91: 1017-1024, 1999.

Feuer EJ, Merrill RM, and Hankey BF: Cancer Surveillance Series: Interpreting trends in prostate cancer B Part II: Cause of death misclassification and the recent rise and fall in prostate cancer mortality. Journal of the National Cancer Institute 91: 1025-1039, 1999.

Etzioni R, Legler JM, Feuer EJ, et al. Cancer Surveillance Series: Interpreting trends in prostate cancer B Part III: Quantifying the link between population prostate-specific antigen testing and recent declines in prostate cancer mortality. Journal of the National Cancer Institute 91: 1033-1039, 1999.

Devesa SS, Grauman DJ, Blot WJ, Fraumeni JF. Cancer Surveillance Series: Changing geographic patterns of lung cancer mortality in the United States, 1950 through 1994. Journal of the National Cancer Institute 91: 1040-1050, 1999.

Linnet MS, Ries LAG, Smith MA, Tarone RE, Devesa SS. Cancer Surveillance Series: Recent trends in childhood cancer incidence and mortality in the United States. Journal of the National Cancer Institute 91: 1051-1058, 1999.

Fraumeni JF, Rimer BK. Commentary: Cancer Surveillance Series: Inauguration. Journal of the National Cancer Institute 91: 1004, 1999.

Wingo PA, Ries LAG, Giovino GA, et al. Annual report to the nation on the status of cancer, 1973-1996, with a special section on lung cancer and tobacco smoking. Journal of the National Cancer Institute 91: 675-690, 1999.

### **Gene For Making Mice Smarter Offers Clue To Human Intelligence**

*Background:* The brain does its work with special message-carrying chemicals. These chemicals, called neurotransmitters, move on pathways inside the brain, and the signals they carry are transformed into colors, sounds, and shapes. These signals tell us if a flower is blue or where we left our car keys, and we use all these perceptions to remember and learn. In order to work properly, different types of neurotransmitters must have their own pre-arranged destinations in the brain, known as receptors—the biological gates neurotransmitters pass through. But what tells neurotransmitters how to find their special receptors? Ultimately, genes, the building blocks of inheritance that provide the overall plan for the body of any living creature.

For some time, scientists have studied the roles of neurotransmitters and their receptors in memory and learning. Recently, research techniques have made it possible to delete or add genes to laboratory mice and determine the effects of the proteins made by certain genes in living brains.

*Advance:* Scientists used a microscopic technique to add an altered gene to mice. This gene alters one type of neurotransmitter receptor, known as an NMDA (N-methyl-D-aspartate) receptor, in a mouse's brain, causing the receptor to better recognize and receive its neurotransmitter, glutamate. The gene produced a component of the receptor that is normally found in young mice, but not adult animals. This alteration is of particular scientific interest because learning abilities decline in many animals as they age. The Ayoung form of the receptor appears to allow a slightly longer time interval for the animal to make an association between 2 events B hence, to learn a connection between the events.

These mice learned faster, remembered more, and were able to apply what they learned. AMouse tests included recognizing new objects and remembering them, and learning to avoid hurting themselves. The scientists named the mice carrying this gene ADoogie (after the popular TV show ADoogie Howser, M.D., about an exceptionally bright young man). In every case the ADoogie mice did better than their littermates who had not received the altered gene.

*Implications:* This is the first time that the alteration of a specific gene can be linked to improved learning in mice. And since the receptor gene the scientists studied is very similar to the same gene in human brains, it now appears that changing a gene to improve human learning abilities might someday be possible. While this insight is still an early development in the biological study of intelligence, it is encouraging news in the fight against age-related memory loss, senility, and Alzheimer's Disease.

Tang Y et al.: Genetic enhancement of learning and memory in mice. Nature 401:63-69, 1999.

## Clues to How our Brains Organize Visual Perceptions

*Background:* Scientists gained much of our early understanding of the functional organization of the brain from observations on brain-damaged patients. Recently, the realization that some brain-damaged patients cannot recognize particular classes of objects, such as faces, suggests that the part of the brain concerned with recognition of objects is organized as a series of object category-specific regions. Brain imaging studies in humans identified regions of the cortex specialized for perceiving faces, landmarks/buildings, and letters, respectively. However, considering the size of these brain regions, it seems unlikely that there could be different regions for all categories of objects, because there are just too many categories and too little cortex. Another possibility is that the representation of an object in the cortex could be more widely distributed than just these specific regions.

*Advance:* A very recent study by NIH intramural researchers seems to have resolved this puzzle. Neuroimaging techniques were used to measure regional changes in brain activation while people viewed images of houses, faces and chairs. Three brain regions within the cortex were found to respond maximally to houses, faces, and chairs, respectively. However, the response to each category of object was not restricted entirely to the region that responded the greatest to that category but, rather, the response also extended to the other regions; thus the response is broadly distributed.

*Implications:* Because images of houses, faces, and chairs, caused a wide expanse of the object-recognition part of the brain's cortex to become activated, it appears that the cortex continuously represents information about the form of objects. Since similar types of objects such as all chairs activate the same regions to the greatest extent, it seems likely that features common to these objects are what the cortical regions are detecting. A greater knowledge of how the brain recognizes and organizes images may help us understand where and how deficits occur in brain-damaged individuals, and, hopefully, lead us to new therapies or treatment strategies for these people.

Ishai A, Ungerleider LG, Martin A, Shouten JL, Haxby JV: Distributed representation of objects in the human ventral visual pathway. Proceedings of the National Academy of Sciences, 96: 9379-9384, 1999.

## **New Brain Cells Formed In Response To Learning**

*Background:* For many years scientists thought the number of brain cells at birth are all we ever have. As we age, it was believed, brain cells progressively die and are never replaced. This idea was frequently offered as the reason behind such age-related problems as memory loss and senility. However, since there was no reliable method to actually observe brain cells over time, there was no way to confirm or deny this idea. About 30 years ago, a new tool was developed for brain research where brain cells known as neurons could be tagged with a chemical that allowed them to be tracked, and it was apparent that new neurons are born throughout the lifetimes of many creatures, including birds, monkeys, and, we now believe, human beings. But if this is true, why do many people still develop age-related problems of memory, thinking, and concentration?

*Advance:* Working with rats, scientists focused on a part of the brain known as the hippocampus, an area associated with learning and memory. The cells of the hippocampus were tagged, and the rats were then given a rigorous training period. The rats learned to navigate in spaces that were new to them, and to associate disconnected events. After the training period, the researchers observed a dramatic increase in the number of neurons in the hippocampus. And more than a week after the training sessions were over, the number of these cells still remained high. Beyond that, the researchers noticed there were more neurons in other areas of the brain as well not just the hippocampus.

*Implications:* Neurons are not only born in response to the challenge of new learning, but live longer when learning is taking place. Learning, practicing, and remembering are all activities that seem to be linked not only to the development of new neurons, but with keeping those new cells healthy, functional, and living longer.

The clear demonstration that learning supports and promotes the health of brain cells is a remarkable addition to our scientific knowledge. After believing that the total number of neurons declined during life (and that how brains are used had no effect on the number of brain cells), we are now understanding that mental challenges impact the number of cells. This work confirms that the brain not only creates new cells throughout a lifetime, but those cells do better live longer and stronger if the brain they are a part of is actively learning.

Aside from giving medical research a strong foundation for understanding problems like learning disabilities and brain damage, this insight tells us that the brain actively responds to needs and demands and, in fact, performs better in the face of challenges. In the largest sense, this research suggests that some aspects of age- or trauma-related memory loss are made worse by a lack of challenges for the brain. It may be that brains need new skills to master in order to maintain clearer thinking and sharper memory for a longer period of time.

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ: Learning enhances adult neurogenesis in the hippocampal formation. *Nature Neuroscience* 2(3):260-265, 1999.

Gould E, Reeves A, Fallah M, Tanapat P, Gross CG, Fuchs E: Hippocampal neurogenesis in adult Old World primates. *Proc Natl Acad Sci USA* 96:5263-5267, 1999.

## How the Brain Pays Attention

*Background:* We are well aware of the normal brain's ability to attend to a given object or task and ignore others when, for example, a student focuses on a math test with all of her concentration. But what about when we recognize a friend's face in a crowd, and don't really see any of the other faces, or when an attractive member of the opposite sex catches your eye? Similar attentional brain mechanisms are probably at play then as well; as they are during virtually all our waking hours, silently enabling us to function better by filtering out the constant bombardment of irrelevant stimuli that the world presents to our senses. Recently, a series of experiments has allowed some insights into where in the brain, and how, this filtering occurs.

*Advance:* Based on findings in non-human primates, NIH intramural neuroscientists were able to test, in humans, whether visual stimuli compete for neural activation by combining clever behavioral paradigms with functional imaging techniques. The researchers found that the brain activations stimulated by different objects in a complex visual scene interact by suppressing each other in ways that, indeed, suggest that the objects compete for brain activation. Further, when the people being tested consciously paid attention to a given object, the effects of the other objects on brain activation were less intense, and that object of attention won the competition.

In a second study, brain activation was measured in subjects who paid attention to a particular location in anticipation of an object that actually did not appear. In this case, the brain was activated in the visual cortex in the absence of visual stimulation. This activation is essentially a neural fingerprint of attention. The researchers were also able to determine which higher cortical areas give rise to such top-down signals related to attention.

A third study, in non-human primates, pinpointed more precisely where along the flow of visual information through the brain, that top-down attentional influences have their effect. Two regions in the temporal lobe known to be involved in object recognition were lesioned, causing the animals to show only mild impairment in a simple perception task. However, when a visual distraction was added, performance of the task by the lesioned animals deteriorated precipitously, indicating impaired attention. These two temporal lobe regions appear to be brain sites where top-down attentional influences can affect the competition for attention among multiple visual stimuli.

*Implications:* Together, these studies have advanced greatly our understanding of how and where higher cortical regions of the brain exert top-down influences on visual stimuli, resulting in attention. Examining attentional control in the human brain may lead to a better understanding of attentional deficits which are common in many brain disorders.

Kastner S, DeWeerd PD, Desimone R, and Ungerleider LG: Mechanisms of directed attention in the human extrastriate cortex as revealed by functional MRI. *Science* 282: 108-111, 1999.

Kastner S, Pinsk MA, DeWeerd PD, Desimone R, and Ungerleider LG: Increased activity in human visual cortex during directed attention in the absence of visual stimulation. *Neuron* 22, 751-761, 1999.

DeWeerd PD, Peralta MR, Desimone R, and Ungerleider LG: Loss of attentional stimulus selection after extrastriate cortical lesions in macaques. *Nature Neuroscience* 2, 753-758, 1999.

## Newly Identified Protein Essential For Message Transmission In The Brain

*Background:* The cells of the brain (*neurons*) transmit messages through a complex series of domino-like interactions. An electrical impulse travels along the long tentacle-like pathway of the neuron to reach the *synapse*, a tiny channel that separates one neuron from another. Here messenger molecules known as neurotransmitters are briefly held in synaptic vesicles, or microscopic bubbles. There are many specific kinds of neurotransmitters, all tailored to be received at particular points (or receptor sites) on the opposite side of the synapse but the transmitter packets must briefly undergo a kind of priming that is necessary before the bubbles are able to fuse to the neuron's surface in a process which then allows their cargo of neurotransmitter to spill into the synaptic opening (known as the cleft), bound for specific receptor sites on the other side where their messages are again converted to electrical impulses for the continuing journey along the fiber pathway of the next neuron in line, all on the way to final conversion in the brain to a specific sensation, perception, reaction, or movement. Specialized protein molecules are needed to encourage the synaptic vesicles-priming process, so that the neurotransmitter can be released. It is here, at the priming point, that scientists have been unsure as to which specific proteins were involved, and in what way these proteins helped the process of neurotransmitter release. This is a vital link knowing which proteins are involved and exactly how they work could lead to impressive advances in developing new medical therapies for brain disorders or trauma.

*Advance:* Using a mouse model, the researchers investigated a protein called Munc13-1 which they believed to be critical in the priming, or maturation, process for vesicles of the brain neurotransmitter called glutamate. By genetically altering a group of mice, they were able to delete the gene responsible for producing Munc13-1. An immediate observation was that the Munc13-1-deficient mice showed a reduction in synaptic responses of 90%. The researchers eliminated a number of possible causes, including direct structural defects as well as electrical transmission problems until, finally, they realized the reduced synaptic sensitivity could be caused by only one of two things: a defect in the release machinery (interference with the release of neurotransmitter from the vesicles they ride in), or a problem on the receiving side of the synapse. In the experiment's second stage the researchers eliminated any difficulties on the receiving, or post-synaptic side of the cleft. This left only one possible answer: Munc13-1 is indeed a critical protein necessary to assist glutamate-laden synaptic vesicles in their fusion-and-release process that is necessary to maintain normal message travel times into the brain.

*Implications:* The scientific team provided direct evidence for a vesicle maturation step in the central nervous system. Prior to this study, proteins facilitating vesicle priming were unknown in the central nervous system, and we had very little knowledge of how synaptic vesicles fuse and release neurotransmitter into and across the cleft. This study is pivotal groundwork for work yet to come, enabling scientists to widen the search for techniques and methods of treating various brain diseases or the results of trauma by improving the transmission of messages into the brain itself.

Augustin I et al.: Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. *Nature* 400: 457-461, 1999.

## Clues to the Nature of Schizophrenia

*Background:* Schizophrenia is a tragic, chronic, and disabling mental illness. This illness typically strikes high-achieving young adults or adolescents suddenly, causing them to have hallucinations and disordered thinking, derailing their plans for education and career, and leading to a difficult and precarious life. A rare, and usually particularly severe, form of the illness begins in childhood and is referred to as childhood-onset schizophrenia, or COS.

We know that a vulnerability to schizophrenia is inherited because the illness tends to run in families. However, scientists are also finding evidence that some non-genetic influences very early in life, perhaps before birth, may also be involved in causing a person to have schizophrenia, even though the illness does not appear until many years later in adolescence or adulthood. This long delay may be related to the progression of normal changes throughout childhood and adolescence in the cells and structures making up the brain, especially the burst of developmental changes that occur during adolescence. In the past, researchers using neuroimaging techniques have found anatomical differences in certain parts of the brains of people with schizophrenia compared to healthy people; however, since these changes appeared after the onset of illness, the relationship to brain development was not clear, but remains an important question.

*Advance:* In a continuing study, NIH intramural researchers are using MRI (magnetic resonance imaging) to examine the brains of children with COS as they mature, and comparing changes in their brain structure with those of healthy children of the same sex and ages. The children with COS were 8-12 years old when they became ill and are now in their mid-to-late teens. MRI scans of the children's brains are being conducted at 2-year intervals. There are clear abnormalities in brain development in the children with COS when compared to the children without COS. The brains of children with schizophrenia show increases in volume in some areas (ventricles) and decreases in other areas (hippocampus and cortex) B areas of the brain important for memory and planning. This pattern of changes is specific to schizophrenia.

*Implications:* It is likely that these progressive changes in the brains of the children with schizophrenia are related to the triggering, or actual onset, of the illness. Research that focuses on these changes may help us to understand the cause and progression of the illness and to develop better medications for treating schizophrenia.

Rapoport JL et al.: Progressive cortical change during adolescence in childhood-onset schizophrenia: A longitudinal magnetic resonance imaging study. Archives of General Psychiatry 56: 649-654, 1999.

## **New Players in the Molecular Basis of Memory and Learning**

*Background:* During learning and memory formation, the connections between neurons, called synapses, change in strength. An increase in the strength of a synapse is called long term potentiation (LTP), while a decrease in the strength of a synapse is called long term depression (LTD). Both LTP and LTD have an early and late phase. The early phase is believed to correspond to short-term memory, which involves temporary, or transient, changes in the proteins located at the synapse. However, in the late phase, believed to correspond to long-term memory, the molecular changes call for the production of new proteins, which requires gene expression & the process of copying, or transcribing, information from DNA to ultimately produce a new protein & this process is modulated by specific other proteins, called transcription factors.

The switch from early to late phase LTP or LTD is an important step in the formation of long-term memories and has been intensively investigated. Many of these studies have shown that the activation of a particular transcription factor, CREB (an abbreviation for cyclic AMP response element-binding protein), is involved in the switch from the early to late phases of LTP. However, it has not been clear whether CREB-regulated gene expression is necessary for LTD as well.

*Advance:* Using both a pharmacological approach and a powerful new technique, called particle-mediated gene transfer, in a nerve cell culture system, NIH-funded investigators examined the role of CREB in LTD. This study showed that the late phase of LTD, like the late phase of LTP, is indeed dependent on CREB activation. As an extension of this work, the researchers looked further upstream in this biochemical pathway to see what activated CREB in LTD. In testing proteins known to activate CREB under other conditions, they found a specific protein called CaMKIV (short for Ca<sup>2+</sup>/calmodulin-dependent protein kinase IV) was also needed for LTD.

*Implications:* This study adds two new and important pieces to our knowledge of how memories are formed. Understanding the molecular basis of learning and memory may help elucidate the causes and treatments of both learning disorders and disease such as Alzheimer's. [secondary B technologies]

Ahn, S., Ginty, D.D., and Linden, D.J.: A late phase of cerebellar long-term depression requires activation of CaMKIV and CREB. Neuron 23: 559-568, 1999.

## Learning How We Learn

*Background:* We constantly adapt and learn from our experiences, whether it be the name of someone we just met, where we parked our car, or the challenging content of a school assignment. But just how do our brains manage to learn this information? It has long been assumed that some change in the brain must underlie the acquisition of memory. In the past years, neuroscientists discovered that synapses, the specialized contacts between brain cells (neurons) where one cell receives chemical signals from another cell, can change to become strengthened with repeated use. This strengthening process is known as long-term potentiation (LTP), while an opposite change, a weakening of connections between synapses, is known as long-term depression (LTD). LTP begins when a specific chemical neurotransmitter, glutamate, released by one neuron, binds to a type of receptor called the NMDA receptor on another neuron. Determining how this binding of glutamate to the NMDA (N-methyl-D-aspartate) receptor, leads to LTP and how LTP relates to memory are intense areas of research.

*Advance:* Two different NIH-funded research teams have now discovered strong evidence of the cooperative role of a second type of glutamate receptor, the AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptor, in the mechanism underlying changes in synaptic strength. The teams demonstrated that AMPA receptors move very rapidly into and out of synapses in association with LTP and LTD. In one study, researchers labeled AMPA receptors with a fluorescent marker. When the nerve cells (neurons) containing the labeled AMPA receptors were visualized with time-lapse two-photon laser scanning microscopy, most of the labeled receptors were found within the interior of the cell. However, when LTP was induced in these neurons, the tagged receptors moved rapidly to the surface of the cell where they aggregated into dense clusters. In the second study, researchers looked at the opposite process by inducing LTD in neurons and then staining the neurons with antibodies to the AMPA receptor. The induction of LTD caused a concurrent decrease in the number of AMPA receptors clustered on the cell's surface. Taken together, these findings indicate that a rapid and specific redistribution of AMPA receptors contributes to LTP and to LTD and point to the regulation of AMPA receptors and their trafficking as the key to our understanding of mechanisms underlying synaptic change.

*Implications:* These studies have provided exciting new knowledge of how our brains begin the learning process. In more general terms, since synaptic transmission is central to almost all brain functions, the studies also show us how subtle alterations in the cellular and molecular mechanisms underlying synaptic change during development and in adulthood might contribute to devastating brain disorders, such as schizophrenia. This knowledge will help guide future research to understand these disorders.

Shi, S-H, Hayashi, Y, Petralia, RS, Zaman, SH, Wenthold, RJ, Svoboda, K, & Malinow R: Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284: 1811-1816, 1999.

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, & Malenka, RC: Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nature Neuroscience* 2: 454-460, 1999.

## Neural Activity Shapes the Brain's Cells

*Background:* Adult brain cells (neurons) come in an enormous variety of shapes. Many cells have long, thin and branching extensions (dendrites) reaching out from the cell body to communicate with other neurons. Signals between neurons are communicated at many sites along these extensions and, since neurons that perform similar functions in the brain have similar shapes (much as the branching patterns of trees of the same species are similar), scientists have suspected that a neuron's branching pattern must be important to the cell's normal functional role. Yet, until recently, very little was known about how these patterns emerge during growth and development of the brain.

*Advance:* Researchers have shown that the activity of neurons may play a profound role in shaping cellular extensions, or dendrites, during brain development. Using cultured rat brain tissues, the researchers labeled a small number of neurons, from a region of the brain known as the hippocampus, with a green fluorescent protein. When the scientists induced synaptic activity at selected sites on the labeled neurons by stimulating receptors for a specific neurotransmitter, changes in shape of the dendrites were observed by time-lapse two-photon laser scanning microscopy. The stimulation of one class of neurotransmitter receptors (*N*-methyl-D-aspartate, or NMDA, receptors) on cell surfaces enhanced the growth of thin, thread-like extensions close to the stimulating electrode and these structural changes were long lasting.

A second team of researchers demonstrated the growth of similar spine-like structures on the surfaces of hippocampal neurons taken from slightly older animals. In these experiments, the new extensions appeared shortly after the induction of long lasting enhancement of functional connections (i.e. long-term potentiation, or LTP).

These findings indicate that neuronal activation within the living brain through NMDA receptors could initiate rapid, input-specific changes in the structure of neurons. Such changes are likely to play important roles, not only in the birth of new connections between neurons, but also in more subtle rearrangements of existing connections.

*Implications:* The findings of this research have fundamental implications for how, at the cellular level, on-going neuronal activity plays a major role in the shaping of the brain's architecture, and hence, its function. Over time, these kinds of changes in cell shape, in response to the actions of specific neurotransmitters, may explain how the young, developing brain shapes its structure. These structural changes may also be involved in important changes in brain cell function that occur during development, such as learning. In addition, if such induced changes are found to occur in adult as well as developing animals, the changes may be involved in adult memory storage, as well.

Maletic-Savatic M, Malinow R, and Svoboda K: Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* 283: 1923-1927, 1999.

Engert F and Bonhoeffer T: Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 399: 66-70, 1999.

## Depressed Mothers= Speech Affects Learning In Babies

*Background:* Becoming depressed after having a baby happens to as many as 15% of women. For those who get postpartum blues, dealing with a newborn can be very difficult. These mothers are like any other depressed person—sad, tired, and not given to playfulness or activity. The healthy growth and development of babies, however, demands their mothers' focused and energetic attention. And while we know that a mother's attention is vital to a child's development, the precise effects of specific forms of attention and stimulation have not been widely studied. An important form of stimulation is speech: how a mother talks to her baby. With that in mind, the scientific team investigated if speech patterns—the tones, sound quality, and musicality—in a mother's voice varied in their effects on the babies of depressed mothers versus the babies of non-depressed mothers.

*Advance:* The team evaluated a group of new mothers for depression using a screening test for this purpose. A group of 21 mothers were found to be suffering with varying degrees of postpartum depression. These women were then tape-recorded while talking to their babies. Later, a group of 4-month old babies (*not* the children of the depressed mothers) were trained to look at a picture—an abstract pattern—while listening to short segments of both the recordings of the depressed mothers as well as recordings of non-depressed mothers.

The babies learned to watch the pattern with interest and focus—but *only* when they were hearing the voices of non-depressed mothers. An infant's inclination to focus on a picture is a learning activity, and the stimulation of a non-depressed mother's voice was clearly associated with the promotion of learning among the babies. What is particularly striking in this study is that speech seems to be what is known as an independent variable, that is, spoken words exerted a significant influence apart from other factors like touch or facial expression. This understanding that a non-depressed mother's voice carries such powerful encouragement for a baby to learn is a striking addition to our knowledge of how human beings successfully grow and develop.

*Implications:* This study demonstrated for the first time that babies react with far less interest to the speech of depressed mothers than they do to non-depressed mothers—that the pitch and tones in a depressed mother's voice do *not* promote attention or learning in a baby. Other information tells us that babies who experience early difficulties in learning often have later problems with behavior and school performance. If we can clearly understand how these early learning difficulties develop, then we can take steps to solve learning and behavioral problems before they start.

This study is also important for its emphasis on recognizing and treating postpartum depression, clearly demonstrating that treatment is helpful to a depressed mother as it preserves their children's abilities to learn. Increased recognition of postpartum depression among health care providers has important and lasting effects for both mother *and* child. [secondary B treatment and prevention]

Kaplan PS, Bachorowski J, Zarlengo-Strouse, P: Child-directed speech produced by mothers with symptoms of depression fails to promote associative learning in 4-month old infants. Child Development 70: 560-70, 1999.

### **Link Established Between Cessation of Cell Divisions and the Mammalian Aging Process**

*Background:* Telomeres are highly repetitive DNA sequences located at the ends of chromosomes. They are essential for the stability of chromosomes and cell survival in a wide variety of organisms. In normal human cells grown in cell culture, telomere length shortens with each cell division and the progressive telomere shortening ultimately limits the ability of cells to divide. However, establishment of a definite link between telomere shortening and aging of the organism requires an experimental animal that lacks telomerase.

*Advance:* Investigators created a mouse model deficient in an enzyme called telomerase in which telomeres progressively shortened throughout the life span of the animal (2.5 years). This **Knockout** mouse provided a unique opportunity to understand the cellular consequences and aging significance of telomere shortening in the living animal. Observations in this model provided a definitive link between telomere shortening and mammalian aging processes. Although loss of telomeres did not elicit a full spectrum of the classical symptoms of aging, age-dependent telomere shortening was associated with a shortened life span, reduced capacity to respond to physiological stresses, and an increased incidence of spontaneous cancers.

*Implications:* As individuals age, aged organs exhibit a markedly diminished capacity to cope with acute and chronic stresses. The telomerase-deficient mouse provides a valuable model to study the role of telomere maintenance in cellular stress responses in the aging organism. This mouse model promises to shed light on a very important aspect of age-related dysfunction.

Rudolph KL, Chang S, Lee HW, Blasco M., Gottlieb GJ, Greider C, and DePinho R: Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96: 701-712, 1999.

### **Caloric Restriction Slows the Aging Process**

*Background:* Most insects and animals exhibit a progressive and irreversible physiologic decline during the aging process. While the molecular basis remains unknown, genetic manipulation of the aging process has been achieved in fruit flies and round worms. In mammals, the only intervention known to slow the intrinsic rate of aging is caloric restriction.

*Advance:* Scientists examined changes in gene expression with aging between young and old mice on calorie-restricted diets. Of the 6500 genes examined, 58 genes showed a two-fold increase of gene expression with advancing age, and 55 genes showed a two-fold decrease in gene expression with advancing age. The increase in expression was seen in genes involved in stress responses and neuronal growth. The major effect of caloric restriction appears to be the induction of a metabolic reprogramming towards increasing expression of genes involved in energy metabolism, and increased protein synthesis and turnover. Of the four major gene classes that displayed consistent age-associated alterations, 84% were either completely or partially suppressed by caloric restriction. This research demonstrated that molecular changes that occur during aging are partially or completely prevented by caloric restriction. Complete or partial prevention of the majority of age-related alterations by caloric restriction suggests that gene expression profiles can be used to assess the biological age of mammalian tissues, providing a tool for evaluating experimental interventions.

*Implications:* This study provides the first global assessment of the aging process in mammals at the molecular level, and underscores the value of large-scale, parallel gene expression analysis in the study of complex biological occurrences. This study suggests that gene expression profiles can be used to assess the biological age of mammalian tissues, providing a tool for evaluating experimental interventions. In particular these results provide evidence that during aging there is an induction of a stress response as a result of damage to proteins and other large molecules.

Lee CK, Klopp RG, Weindruch R, and Prolla TA: Gene expression profile of aging and its retardation by caloric restriction. Science 285: 1390-1393, 1999.

## Gene Therapy Can Maintain Muscle Mass and Strength

*Background:* -During the aging process, mammals, including humans, lose up to one-third of their skeletal muscle mass and strength. In humans, this loss occurs gradually between the ages of 30 and 80. Insulin-like growth factor 1 (IGF-1) is a protein that is responsible for mediating the growth and maintenance of skeletal muscle as well as other tissues. With aging, IGF-1 levels decline in skeletal muscles. This change is exhibited by an age-related loss of muscle mass and function.

*Advance:* To test whether muscle-specific expression of IGF-1 can attenuate or prevent age-related loss of muscle mass and strength without affecting other parts of the body, scientists modified the IGF-1 gene to ensure that it would only be expressed in muscle. As a second safeguard, the gene was injected directly into the muscle. From a functional standpoint, the effects of IGF-1 overexpression in skeletal muscle were very dramatic. Two important hallmarks of aging muscle were prevented by IGF-1 administration: 1) IGF-1 expression increased the size of existing individual muscle fibers with concomitant increases in muscle force in the aged mice; and 2) IGF-1 expression completely prevented the loss of powerful muscle fiber types seen in aging skeletal muscle. This study demonstrated that expression of the (IGF-1) gene in the skeletal muscle tissue of old mice reduces age-related loss of muscle mass and function by stimulating growth and increasing strength of existing muscle fibers.

*Implications:* The results of the muscle IGF-1 gene transfer studies in mice suggest that muscle-specific delivery of IGF-1 could form the basis of a human gene therapy for preventing or reversing the loss of muscle function associated with aging and functional impairments due to muscle diseases. [secondary B treatment]

Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, and Sweeney HL: Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. Proc. Natl. Acad. Sci, USA 95: 15603-15607, 1998.

Musaro A, McCullagh KJA., Naya FJ, Olson EN, and Rosenthal N: IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-ATc1. Nature 400: 581-585, 1999.

### **Does the Relationship between Health and Economic Status Reverse Over the Life Course?**

*Background:* There is a striking and well-documented relationship between socioeconomic status, health, and longevity. People with higher incomes and more wealth tend to be healthier and to live longer. The causes of this relationship are largely unknown. The effect of positive health behaviors and access to medical care are insufficient to explain individual health outcomes. Instead, some intriguing competing theories have arisen that emphasize long-term impacts of early childhood, the cumulative effects of prolonged exposures to individual stressful events, or reactions to macro-societal factors such as rising levels of income inequality. Economists are now making contributions to understanding of the alternative pathway--the impact that poor health has on economic resources.

*Advance:* In middle and older ages, there are pronounced effects of new health shocks on household income and wealth, but it is an open question how much earlier in the life cycle such a sweeping statement is true. While economic resources also appear to impact health outcomes, this may be most acute during childhood and early adulthood when health levels and trajectories are being established. The strong inter-relationship between wealth and health at older ages may be due to adverse health events that have adverse economic implications. People who have severe health events have much larger reductions in total wealth than their medical expenses would suggest. The larger reductions in wealth appear to be caused by reduced earnings that stem from taking early retirement or other reductions in work. People who have heart attacks, strokes, or other acute health events that cause declines in functional ability appear especially likely to reduce their work levels. There is just as large a reduction in wealth among those with and without health insurance (although those with health insurance have lower out-of-pocket medical expenses), suggesting that health insurance does not fully protect people from the economic costs of adverse health events.

*Implications:* The finding that adverse health events have significant economic consequences is an important component to unraveling the difficult questions of why socioeconomic status and health are so strongly related. It demonstrates how differences in health status can cause differences in economic circumstances. These results also suggest some direction for policy. They show, for example, that health insurance deals with only a small part of the economic cost of declining health. The much larger economic costs of decreased work and lost earnings might be more effectively addressed in other ways.

Smith, JP: Healthy bodies and thick wallets: the dual relation between health and economic status. Journal of Economic Perspectives 13 (no. 2 Spring): 145-66, 1999.

### **Protein Complexes in Cells Can Use Energy to Promote Subsequent Function or Loss of Function**

*Background:* The cell nucleus contains a mixture of soluble proteins that interact with each gene to turn it on or off. Recently, it has been found that gene function can also be enhanced or repressed by protein complexes that work on larger gene regions. In the cells of lower organisms and in human cells, the nuclear complexes have been classified into Apositive@ complexes that activate regions and Anegative@ complexes that repress them. Positive complexes use the energy of ATP to bind to and reorganize gene regions (a process called Achromatin remodeling@). A specific protein (MTA1) in the protein complex, NURD, has earlier been associated with cancer metastasis. Negative complexes have been thought to act directly on existing structures, making chemical modifications that tighten a gene region into a relatively inert form.

*Advance:* A novel human protein complex, named NURD, has been recovered that opens up gene regions, making the genes accessible to that repress gene expression. The repressive activity (Ahistone deacetylase@ activity) is stimulated by ATP in artificial chromatin studied outside of cells, suggesting that chromatin remodeling helps the deacetylase to get to its sites of action. The results suggest that ATP-dependent chromatin remodeling can participate in repression of gene regions as well as in activation, by assisting repressive activities to gain access to sites of action. Thus, gene function can be enhanced (activated) or turned off (repressed) by protein complexes which move from one chromosomal region to another in the cell nucleus.

*Implications:* The involvement of chromatin remodeling in gene function is itself fundamental, but it is also increasingly implicated in other biological processes. For example, helicase proteins found in chromatin-remodeling complexes are implicated in premature aging syndromes, and the NURD component MTA1 is associated with cancer progression. The numbers of such complexes, as well as their interactions with other nuclear components, are just becoming known; but it is now clear that ATP-dependent chromatin remodeling is generally part of the driving force for both positive and negative control of gene expression.

Xue Y, Wong J, Moreno GT, Young MK, Côte J, and Wang W: NURD, a Novel Complex with Both ATP-Dependent Chromatin-Remodeling and Histone Deacetylase Activities. Molecular Cell 2: 851-861, 1998.

## Control of Programmed Cell Death in Human Tumor and Immune Cells

*Background:* Apoptosis (programmed cell death) is an important physiological process that ensures the elimination of damaged or unwanted cells in healthy individuals. Aberrant regulation of cell death (either too little or too much) contributes to the development of many disorders, including Alzheimer's disease, AIDS, rheumatoid arthritis, and cancers. The action of therapeutic agents (chemotherapy) to destroy tumor cells can be rendered ineffective by the family of anti-apoptotic proteins (Bcl-2 and Bcl-X<sub>L</sub>). The precise mechanism of action of these proteins in preventing apoptosis is poorly defined. The research described here examined and characterized the molecular mechanisms of Bcl-protein activity in human tumor and immune cells.

*Advance:* The family of Bcl proteins inhibit cell death which otherwise could be induced by a variety of stimuli, including chemotherapeutic agents. The standing hypothesis regarding the function of the Bcl-2 family of proteins is that they bind to other proteins that would promote programmed cell death, thus preventing the death of those cells. Recent laboratory research has documented that Bcl-2 proteins can overexpress, making tumor cells resistant to anti-cancer agents. The effects of Bcl-2 can be, at least partially, overcome by bathing tumor cells with high doses of chemotherapy. This treatment results in inactivation of Bcl-2 proteins.

Understanding the role of Bcl-2 in stopping or delaying apoptosis may lead to clinical applications. Illnesses related to early cell death, such as Alzheimer's disease, may be correctable by inducing expression of Bcl-2. Experiments are underway to evaluate whether mutant cells containing modified Bcl-2 proteins that cannot be turned off can protect blood-cell producing stem cells against high chemotherapy doses.

*Implication:* Careful exploration and identification of the biochemical pathways and action of proteins that can prevent or allow programmed cell death should lead to development of new methods of promoting cell death in malignant tumors and other cell-overgrowth disorders, as well as preventing early cell death in such illnesses as Alzheimer's disease. [secondary B treatment]

Srivastava RK, Sasaki CY, Hardwick MJ and Longo DL: Bcl-2 mediated Drug Resistance: Inhibition of Apoptosis by Blocking Nuclear Factor of Activated T Lymphocytes (NFAT)-induced Fas Ligand Transcription. J. Exp. Med. 190: 253-266, 1999.

Srivastava RK, Sollott SJ, Khan L, Hansford R, Lakatta EG, and Longo DL: Bcl-2 and Bcl-X<sub>L</sub> block thapsigargin-induced nitric oxide generation, c-jun NH<sub>2</sub>-terminal kinase activity and apoptosis. Mol. Cell. Biol. 19:5659-5674, 1999.

Srivastava RK, Mi QS, Hardwick JM, and Longo DL: Deletion of the loop region of Bcl-2 completely blocks paclitaxel-induced apoptosis. Proc. Natl. Acad. Sci. 96: 3775-3780, 1999.

### **Gene on Chromosome 13 Linked to a Form of Familial Early-Onset Dementia**

*Background:* A form of dementia in a large British pedigree that spans seven generations has been linked to a newly discovered, dominant gene, BRI, on chromosome 13. Familial British dementia (FBD) is characterized by progressive dementia, spasticity and cerebellar ataxia, and has an age of onset of approximately 50 years of age. Abnormal protein deposits including cerebral amyloid angiopathy, plaques in the vicinity of blood vessels, and neurofibrillary tangles are the predominant pathological lesions. FBD is similar to Alzheimer's disease because in both disorders the production of a small insoluble peptide from a transmembrane protein is a key feature. Further, the neurofibrillary pathology observed in both FBD and AD is identical. The mutation causes a slightly longer than normal protein to be formed, and it is that extra segment together with the end portion of a normal protein (Bri protein) that is clipped out and forms amyloid.

*Advance:* This research resulted in the discovery of a new genetic defect that causes an early-onset form of dementia that is similar, at least neuropathologically, to AD. While much remains to be accomplished with respect to investigating the BRI gene and the function of the protein that it produces, it is expected that understanding how the gene defect causes the disease will lead to insights into the pathogenesis of other neurodegenerative diseases characterized by amyloid A deposition as well.

*Implications:* In a manner analogous to the mutations of amyloid precursor protein, which enhance the clipping-out of the A $\beta$  peptide, the mutation in the BRI gene seems to result in the release of an amyloidogenic ABri subunit. Understanding how the genetically different disorder (FBD) develops will support efforts to understand the development and progression of the more prevalent Alzheimer's disease. Further, insights gained in FBD may be informative for the design and development of treatments intended to disrupt peptide aggregation and prevent the ensuing neurodegeneration not only in FBD and AD but also in the prion diseases, which are caused by infectious particles called prions. [secondary B treatment]

Vidal R, Frangione B, Rostagno A, Mead S, Révész T, Plant G, and Ghiso J: A stop-codon mutation in the *BRI* gene associated with familial British dementia. *Nature* 399: 776-81, 1999.

**Two Amino Acid Residues are Critical to Presenilin Protein Activity:  
Leads to Potential Targets for Treatment of Early-Onset Alzheimer's Disease**

*Background:* It is known that most early-onset Alzheimer's disease (AD) is the result of a large number of mutations in one or the other of human presenilin (PS) genes, but just how those mutations result in the disease is not known. Early on, it was determined that a protein, *sel-12*, in a nematode (roundworm) was similar in sequence to the PS proteins. *Sel-12* is involved with cellular signaling and cell fate decisions in both the worm and the mouse. The importance and immediate relevance of PS function to AD rests on the observation that every known PS mutation affects the processing of amyloid precursor protein (APP) and thereby the generation of its  $\beta$ -amyloid fragment. The biochemistry and pathophysiology of the PS proteins is being intensively investigated in a number of model systems. Recent research focusing on the function of PS-1 protein in human and in lower organisms has suggested that either PS-1 and PS-2 have protein clipping activity or that the PSs function as co-factors to enhance the activity of some other protease that clips APP.

*Advance:* Mutations of either of two amino acid (each an aspartate) residues in the presenilin-1 protein have been found to result in a substantial reduction in  $\beta$ -amyloid production and an increase in the amount of terminal fragments of  $\beta$ -amyloid precursor protein that, essentially, stop amyloid production. When the sequence of amino acids of the presenilin protein was altered from its normal sequence in two critical locations, buried within the cell membrane, amyloid formation was reduced. That suggested that these two residues were crucial for clipping-out the amyloid fragment from the precursor protein. That role or activity, heretofore, has been associated with a specific but unidentified enzyme called  $\gamma$ -secretase. Although it is too soon to know for certain, these results suggest that PS-1 may be able to clip the  $\beta$ -amyloid fragment from APP, despite the fact that the sequences of amino acids in PSs bear no sequence homology to other known proteases.

*Implications:* This result has provided new insights to the potential interaction of two key molecules (the amyloid precursor protein, APP, and presenilin protein, PS) involved in amyloid formation. These studies could have implications for the treatment of AD and related disorders of amyloid accumulation. Significant advances in therapeutics research targeted at reducing  $\beta$ -amyloid generation and plaque formation are possible based on the leads provided by this research. [secondary B treatment]

Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, and Selkoe DJ: Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and  $\gamma$ -secretase activity. *Nature* 398: 513-17, 1999.

## **New Neurons Are Produced in the Adult Human Brain**

*Background:* Neurons and glial cells, the other major cell type found in the brain, are formed from neural stem cells in fetal tissue and in tissue lining the brain ventricles in postnatal animals. Limited generation of neurons and neural stem cells has been demonstrated in selected brain regions of adult rodents, including the hippocampus, a region involved in learning and memory.

*Advance:* Researchers have found that mice reared in an enriched environment that included physical activity, social interaction, and learning opportunities exhibited an increased number of neurons in the hippocampus. Of the various enrichment factors, it was found that physical activity in the form of voluntary wheel running, but not swimming, increased cell reproduction, cell survival and neurogenesis in the brain hippocampal region.

In humans, scientists have now discovered that the adult brain retains an ability to generate neurons throughout life. Reproducing brain cells were identified by the injection of a compound, BrdU, which is incorporated into DNA as cells divide. Cancer patients were given the BrdU for diagnostic purposes. Examination of postmortem brain tissue obtained from these patients (average age at death was 64 years) revealed the presence of BrdU labeled neurons and glial cells in the hippocampal region. Thus, the brains of adult humans contain dividing neural stem cells, which can generate neurons and glial cells, in a region important for learning and memory. The results suggest that a form of brain plasticity involving the addition of new cells continues throughout the human life-span.

*Implications:* The results of the physical activity studies in rodents are intriguing because they suggest that specific components of complex behaviors can modify the structure and, presumably, function of the adult brain, and that this behavior modification might be able to alleviate age-related decline in brain function by generating new neurons.

In humans, the finding that neurogenesis occurs in adult brain has great impact for aging research. It may be possible to stimulate intrinsic brain repair mechanisms to replace neurons and glial cells lost through age, trauma, and disease, including neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases as well as age-related cognitive decline. Finding ways to stimulate the formation of new brain and spinal cord neurons might lead to novel therapeutic approaches for brain injury as well. Isolation of adult brain stem cells, already underway in some laboratories, may provide an ideal source of cells for transplantation and gene therapy strategies in the treatment of neurodegenerative diseases. [secondary B treatment]

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, and Gage FH: Neurogenesis in the adult human hippocampus. Nat. Med. 4: 1313-17, 1998.

Kempermann G and Gage FH: New nerve cells for the adult brain. Scientific American 280: 48-53, 1999.

van Praag H, Kempermann G, and Gage FH: Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuroscience 2: 266-270, 1999.

## **Age Does Not Change the Ticking of the Circadian Clock, but it's Faster than We Thought**

*Background:* The daily cycle of sleep and wakefulness is related to the 24-hour solar day. It has long been believed that the human circadian clock had a period of about 25.25 hours, and that it speeds up when we grew older. This concept of the shortening of the period with age was used to explain the common observation that many older people went to sleep earlier in the evening and awoke earlier in the morning. This pattern, the circadian rhythm, is controlled by a small group of neurons deep within the brain, the suprachiasmatic nucleus of the hypothalamus. Circadian rhythms are pervasive throughout plants and animals, and are tightly entrained to the solar rhythm. Even in the absence of light, these circadian rhythms are maintained and cycle with a period (duration) close to 24 hours. This "free-running" period is genetically determined and varies very little within individuals of the same species. However, earlier studies had reported that the free-running circadian rhythms of humans were longer, about 25 hours, more variable between individuals, and shortened with age. A new study, however, questions these findings and indicates that the human circadian clock is more similar to that of other species and is stable throughout adult life.

*Advance:* It now has been reported that the human circadian clock has a period of close to 24 hours (24 hr 11 min), similar to other species, rather than about 25 hours as previously thought. This study used a technique that controls the confounding effects of entrainment (resetting) of the circadian clock by light and other non-light synchronizers. This study found no difference in circadian period between healthy young and older individuals. This counters the belief that the circadian clock speeds up (shortens in duration) as we age. These findings indicate that the human circadian clock is as stable and precise as that of other animals. It suggests that the new findings of the molecular and genetic mechanisms regulating the circadian clock of other species also may apply to humans.

*Implications:* This study changes some fundamental assumptions about the causes of sleeplessness among the elderly. Poor sleep is not a function of being old by itself. Other factors associated with aging, such as disease, changes in environment, or concurrent age-related processes may contribute to problems of sleep on older persons. Furthermore, the stability of the circadian clock across the adult lifespan indicates that precise circadian timing is vital to the health and well being of humans. Finally, the similarity of circadian periods across the animal kingdom suggests that the findings of basic cellular and molecular mechanisms in these model systems will be applicable to solving the problems of sleep and wakefulness in humans.

Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, Ronda JM, Silva EJ, Allan JS, Emens JS, Dijk DJ, and Kronauer RE: Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177-81, 1999.

## **Synchrotron Resources Enable Landmark Studies of Ribosome Structure**

*Background:* Proteins are known as the workhorses of the cell, for they perform most of the molecular activities essential to life. To function properly, proteins must first be assembled according to explicit molecular blueprints, and this critical activity occurs within a complex subcellular "factory" known as the ribosome. A mammalian cell typically contains some ten million ribosomes, which are each composed of a large and a small subunit. Scientists have long struggled to get a glimpse of these tiny protein factories, and techniques such as advanced microscopy and nuclear magnetic resonance spectroscopy have provided broad outlines of the ribosome's three-dimensional architecture. However, high-resolution images have remained elusive, in part because ribosomal subunits proved too large and complex to be analyzed by x-ray crystallography.

*Advance:* Using the high-energy x-ray light that is available only from synchrotrons, two independent teams of investigators probed crystals of the large and small ribosomal subunits from bacteria and determined their three-dimensional structures in unprecedented detail. Both studies were performed with assistance from the macromolecular crystallography resource at the National Synchrotron Light Source, Brookhaven National Laboratory. The structures were each solved at a resolution of about 5 angstroms--a measurement that is about one ten-thousandth the thickness of a human hair--which allowed the researchers to differentiate particular protein and RNA components of the ribosomal subunits and also gain a better understanding of how the contours of the large and small subunits nestle together to form a complete ribosome.

*Implications:* These new structural studies of the ribosome, coupled with other synchrotron-supported analyses now under way, represent a significant breakthrough toward understanding how ribosomes manage to decipher molecular blueprints and meticulously assemble protein chains. On a practical level, knowledge of ribosome structure may also expedite discovery of effective antibiotics through rational drug design, since antibiotics often work by inhibiting ribosome function in bacterial cells. [secondary B treatment]

Ban N, Nissen P, Hansen J, Capel M, Moore PB, and Steitz TA: Placement of protein and RNA structures into a 5 A-resolution map of the 50S ribosomal subunit. Nature 400:841-7, 1999.

Clemons WM, May JLC, Wimberly BT, McCutcheon JP, Capel MS, and Ramakrishnan V: Structure of a bacterial 30S ribosomal subunit at 5.5 A resolution. Nature 40:833-40, 1999.

### **HIV Infection Persists Even With Combination Drug Therapy**

*Background:* Human immunodeficiency virus (HIV) infection can be controlled with combination drug treatments, which delay disease progression and prolong survival. Mathematical modeling of virus levels has predicted that two or more years of combination therapy might completely eliminate HIV from the body, but more recent reports suggest that a reservoir of viable HIV may persist in some cells, which would necessitate indefinite continuation of combination therapy to keep the virus in check.

*Advance:* Scientists at Northwestern University Medical School used sensitive indicators of viral activity to test blood samples from five HIV-infected patients who were undergoing combination therapy and who had previously undetectable levels of HIV in their blood for 20 months or more. The sensitive tests confirmed the presence of persistent reservoirs of viable HIV, even after two or more years of combination therapy. The persistence of this infected cell population and incomplete suppression of viral replication--not previously predicted by mathematical models--mean that mathematical predictions of viral eradication are not yet reliable.

*Implications:* Combination drug treatments are complex, expensive, and difficult for patients; however, it was hoped that the therapies would eventually eliminate the HIV from the body. The current study indicates that HIV may not be eradicated with current treatments; therefore, improved models of HIV infection and new treatments must be pursued. [secondary B treatment]

Furtado MR, Callaway DS, Phair JP, Kunstman KJ, Stanton JL, Macken CA, Perelson AS, Wolinsky SM: Persistence of HIV-1 Transcription in Peripheral-Blood Mononuclear Cells in Patients Receiving Potent Antiretroviral Therapy. New England Journal of Medicine 340:1614-22, 1999.

## **Nuclear Magnetic Resonance Reveals More Pieces of the Prion Puzzle**

*Background:* Prions are proteins normally found in the brains of all animals, but aberrant forms of the proteins are associated with deadly neurodegenerative diseases that leave the brain riddled with holes. Creutzfeldt-Jakob disease (CJD), the human prion disorder, can be acquired by eating tainted beef, and prion diseases in animals include scrapie in sheep and bovine spongiform encephalopathy (BSE, or mad cow disease) in cows. Not all prion diseases are infectious, as some cases of CJD occur sporadically and others are inherited. A wealth of data suggests that prion diseases arise when the three-dimensional structure of the normal cellular prion protein is converted--through an unknown mechanism--into an infectious pathogenic form.

*Advance:* One avenue to understanding the cause of these infectious, deadly diseases is to identify the structural changes that convert a normal protein to one that causes disease. To study such a modification, researchers have employed nuclear magnetic resonance (NMR). NMR detects signals from carbon atoms, and the signals differ depending on how many hydrogens are in the vicinity. Integrating the data provides structural information. New high-field NMR spectrometers allowed researchers to identify the crucial parts of the prion protein structure that can change and cause infectious or rare inherited disease. Individuals with positively charged amino acid residues in the prion protein do not get prion diseases.

*Implications:* Powerful NMR instruments allowed investigators to gain entirely new insights into the prion protein, which now opens the possibility of breeding scrapie-resistant or BSE-resistant herds. The economic and political consequences of prion diseases such as mad cow disease, and the public fears these diseases often engender, add to the urgency of understanding the biochemical bases of prion disorders. Knowledge of the structural changes that produce the disease-causing proteins may also shed light on other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, both of which are associated with abnormal aggregations of proteins, small peptides, or protein fragments in the brain.

Liu H, Farr-Jones S, Ulyanov NB, Llinas M, Marqusee S, Groth D, Cohen FE, Prusiner SB, and James TL: Solution structure of syrian hamster prion rPrP (90-231). Biochemistry 38:5362-77, 1999.

### **AIDS Virus Strains in Africa: Novel and More Virulent**

*Background:* A large number of distinct AIDS virus strains have been identified in Africa where the disease has been most devastating. Each strain may have different characteristics. For example, some strains may be capable of spreading faster among people, while other strains might prefer to infect different cells in the body or may cause much more damage to the immune system. Therefore, Africa has provided an ideal setting for studying how the different AIDS virus strains infect people and affect their health over time.

*Advance:* An NIH-funded study found four different AIDS virus strains in a group of infected women living in Nairobi, Kenya. Viruses that appeared to be a hybrid of two different strains were found in a significant number of women. This finding suggested that new hybrid versions of the AIDS virus could be very common in Kenya and other parts of Africa where there are a large number of people infected with AIDS. One virus strain in particular was found to be the most damaging to women.

*Implications:* Understanding the differences among the strains of AIDS viruses will be critical to decisions on how to develop and use anti-AIDS vaccines. This study shows that women in Kenya who are infected with one particular AIDS virus strain are at greater danger than women infected with the other strains present in the population. A long-term study is needed to determine how often each strain infects people and how rapidly it damages their immune systems.

Neilson JR, John GC, Carr JK, Lewis P, Kreiss JK, Jackson S, Nduati RW, Mbori-Ngacha D, Panteleeff DD, Bodrug S, Giachetti C, Bott MA, Richardson BA, Bwayo J, Ndinya-Achola J, and Overbaugh J: Subtypes of Human Immunodeficiency Virus Type 1 and Disease Stage among Women in Nairobi, Kenya. Journal of Virology 73: 4393 - 4403, 1999

### **Cooking Fuel, Indoor Pollution and Tuberculosis in India**

*Background:* Tuberculosis is the leading killer of adults worldwide. Recent estimates from the World Health Organization indicate that 44 % of people in Southeast Asia are infected with Tuberculosis. Even discounting the growing epidemic of AIDS in India which has contributed to a resurgence of tuberculosis, this disease is responsible for the deaths of approximately one half a million people in India annually. Cooking smoke is a known risk factor for a number of respiratory diseases. Approximately three-quarters of all households in India use wood or dung as the primary cooking fuel. This type of fuel is high on the scale of pollution and low on the scale of fuel efficiency. In addition, cooking areas in many Indian households are poorly ventilated. Since many women spend much of their time in this area they are especially vulnerable to the effects of indoor air pollution. This study examined the risks for pulmonary tuberculosis among people who use unprocessed cooking fuels as their primary source in their homes.

*Advance:* Data for this study was obtained from a recent health survey in India. Questions included health status of family members, gender, housing type, type of fuel used for cooking, urban or rural location, education, and caste. The results show that exposure to cooking smoke substantially increase the risk of active tuberculosis among people aged 20 and older. Rates for active tuberculosis were significantly higher among those living in households using unprocessed fuels than among those living in households using cleaner fuels. The effect of these fuels appears to play a significant role in the development of tuberculosis for both men and women but the effect is much greater for women. Unprocessed fuels are used more frequently in rural areas, accounting for the increased prevalence of tuberculosis in these regions.

*Implications:* Tuberculosis is a disease that has a particularly onerous burden because it affects adults in their prime. Not only does this have economic implications for the workforce but the consequences on their dependants are also significant. This data suggests that the prevalence of tuberculosis in India, and in other developing countries, could be reduced by a shift from the use of unprocessed fuels to cleaner fuels. For the near future, a more feasible policy might be for the government of India to increase its efforts to educate the public about the adverse effects of cooking smoke. Other options include subsidizing the cost of inexpensive stoves that are fuel efficient and focusing on improving the design with respect to the way the pollutants are released from the stove.  
[secondary B prevention]

Mishra VK, Retherford RD and Smith KR. Biomass Cooking Fuels and Prevalence of Tuberculosis in India. International Journal of Infectious Disease 3:119-129, 1999

### **Lung Disease in Rice Granary Workers**

*Background:* Exposure to grain dusts has long been known to cause a variety of respiratory conditions including asthma, bronchitis and A grain fever<sup>®</sup>. The symptoms of grain fever include fever and/or chills, muscle pains and complaints related to the upper airways and lungs a few hours after exposure to grain dust. While the health effects of dust of the major grains cultivated in the West have been studied, little attention has been paid to the respiratory health effects of exposure to rice dust. Since rice is a major grain staple in China, a large number of farmers and grain handlers are exposed to rice dust.

*Advance:* This study shows that that rice-granary workers experience chronic respiratory responses similar to those of workers exposed to the dusts of sorghum, barley and other grains cultivated in the western world. An investigation was done to look at respiratory symptoms and lung function in rice handlers and processors from granaries in rural areas near Shanghai, China. Workers without exposure to grain dust were used as a reference population. Granary workers reported a significant excess of acute and chronic symptoms including cough sputum production, chronic bronchitis, A grain fever<sup>®</sup> as well as nasal and skin irritation. Impaired lung function tests were also seen in those who reported A grain fever<sup>®</sup>.

*Implications:* Exposure to multiple risk factors including smoking and other indoor and outdoor pollutants appears to be much higher in the developing than in the industrialized world. Dust exposure is one factor that seems to exacerbate other lung diseases such as chronic obstructive lung disease. Greater attention should be paid to occupational exposures B in this case exposure associated with the handling and processing of grains. As chronic disease impacts on greater numbers of people in China and in other developing countries, with the demographic transition to a lower fertility rate and a decrease in infectious diseases, understanding of the role of occupational exposure provides possibilities for prevention. Rice granary workers should be apprised of their risk, and consideration should be given to providing improved ventilation or protective respirator equipment. [secondary B prevention]

Ye TT, Huang JX, Shen YE, Lu P and Christiani DC. Respiratory Symptoms and Pulmonary Function among Chinese Rice-granary Workers. International Journal of Occupational and Environmental Health 4: 155-159, 1998

### **Gene Therapy Restores Muscle in Aging Mice**

*Background:* Muscle strength decreases up to one-third in humans between ages 30 and 80. The risk for falls increases with age, and with less muscle cushioning the vertebrae and the hip area, the impact of a fall is taken much more directly by the bones.

*Advance:* Researchers (supported by NIH and the Muscular Dystrophy Association) are using gene therapy to help the body fight the seemingly inevitable effects of aging or to give it a hand in repairing the damage caused by injury or muscle-wasting disorders like muscular dystrophy. Using a virus to carry a growth-promoting gene directly to muscle fibers, the scientists were able to prevent in mice the age-related decrease of muscle size and strength that leads to unsteadiness and impaired mobility. Normally, when muscle is damaged, satellite cells within the muscle are activated to do their repair work by insulin-like growth factor-1 (IGF-1) and other signaling proteins. In elderly humans and animals, the ability of muscle to activate satellite cells to repair muscle mass diminishes. In the case of muscular dystrophy, muscle damage occurs at such a high rate that the body's intrinsic repair system can't keep up. These researchers have addressed the weakened repair system by putting back into the muscle a chronic signal that would keep the satellite cells activated to be more responsive and repair damage more completely. While the method of delivery is similar to other forms of gene therapy, the aim is somewhat different -- increasing production of a substance that has been appearing in the cells all along. Much gene therapy is aimed at repairing a defective protein or fighting an invading organism.

*Implications:* These findings have potential applicability for a broad range of people including those who have suffered a bad burn or severe muscle tears from sports injuries, those on missions in space or immobilized for an extended period because of an accident, and those who have particular muscular dystrophies such as Becker's because it may lead to treatments for these disorders. [secondary B treatment]

Barton-Davis, et al. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. PNAS 95:15603-15607, December 1998

## Nitric Oxide Perfusion in Patients with Sickle Cell Disease

*Background:* Sickle cell anemia is one of the most prevalent inherited disorders. Significant problems in a number of organs, including the lungs, can occur when there is sickling of the red blood cells, caused by a number of things including low levels of oxygen in the blood, dehydration, and infection. These sickled cells can alter blood flow in blood vessels, and in the most extreme circumstances this can lead to premature death. This severe sickling with organ damage produces sickle cell Acrisis.<sup>@</sup> Nitric oxide is a naturally occurring gas in the body that has effects on the walls of blood vessels, particularly in the lung, and, in addition, it can bind to hemoglobin and change its structure. This potentially beneficial property may allow the nitric oxide to be delivered and released in vital structures, such as the lung, leading to an effect on blood vessel walls producing dilation and improved blood flow. This therapeutic delivery of nitric oxide may be beneficial to patients with sickle cell anemia who have impaired blood flow in small blood vessels because of a direct effect on vasodilation.

*Advance:* Scientists have discovered that nitric oxide given to individuals via an inhalation delivery system can definitely bind to hemoglobin, and that there is a step-wise increase in the amount of binding after exposure to increased amounts of nitric oxide. This binding of nitric oxide to hemoglobin is beneficial because it facilitates transport of nitric oxide to the most distal blood vessels in the body. At the highest levels of nitric oxide delivery, there was also a significant difference in the amount of nitric oxide bound to hemoglobin in the arterial circulation compared to the venous circulation. This progressive increase in the binding to hemoglobin, as well as the higher levels in the arterial circulation, may provide a mechanism to augment nitric oxide transport to the small blood vessels and possibly improve blood flow. Ongoing work is focused on determining the extent of this binding and determining the exact binding site of the nitric oxide on the hemoglobin molecule.

*Implications:* Although these experiments represent preliminary work, they do provide evidence of a possible new approach for treating sickle cell anemia. By improving blood flow in small blood vessels with nitric oxide, vascular damage to the lungs due to sickling cells may be lessened preventing pulmonary failure, the need for patient support with mechanical ventilation, and patient death. Nitric oxide has to be delivered by an inhalation system, but new chemical compounds have been and are being developed which may facilitate delivery of nitric oxide via oral therapy. These new Anitric oxide donors<sup>@</sup> are being studied in animals, and if safety is validated, then they may be studied in humans. An Aoral form<sup>@</sup> of nitric oxide would be easier and more practical to give to patients. These potential new therapies may result in improvement in the quality of life of patients with sickle cell disease and may also lead to health-related cost savings by either preventing or limiting hospital stays for patients with sickle cell disease who have a Acrisis.<sup>@</sup> [secondary B treatment]

Gladwin MT, et al.: The acute chest syndrome in sickle cell disease: role of nitric oxide in its pathophysiology and treatment. American Journal of Respiratory and Critical Care Medicine. 159: 1368-1376, 1999.

Gladwin MT, et al.: Inhaled nitric oxide augments nitric oxide transport on sickle cell hemoglobin without affecting oxygen affinity. Journal of Clinical Investigation. In Press, October, 1999.

## **Evidence that Alcohol Has A Docking Sites® on Cells Raises Potential for New Medications**

*Background:* 14 million adult Americans are alcoholics, and alcohol disorders cost the Nation an estimated \$166 billion annually. Researchers are seeking medications to prevent and treat this common, costly disease. To design more effective medications for alcoholism, however, scientists must identify sites where alcohol acts on cells. Researchers are examining how alcohol depresses nerve cells, which thus dampen the command center -- the nervous system -- that regulates everything from movement to the thinking functions that make humans unique.

Nerve cells transmit impulses to and from each other electrically, with the help of negatively and positively charged particles called Ions.® Surrounding nerve cells are fatty membranes that protect the cells' contents and act as conduits for substances that enable cells to do their work. Proteins embedded in the membrane act as channels (ligand-gated ion channels or LGICs®) that permit only certain ions in or out, creating the right conditions for nerve cells to carry impulses. LGICs are complex structures made of many proteins that together regulate the flow of ions in and out of the channel. For example, when the neurotransmitters (the chemical messengers of the nervous system) GABA or glycine bind with a protein within the LGIC, the channel allows ions in, inhibiting electrical conduction in nerve cells. Alcohol interferes with LGICs in a way that increases ion flow, further inhibiting electrical conduction.

Does alcohol work directly on LGIC proteins embedded in the membrane, or does it dissolve the fatty portion of the membrane, disrupting the proteins' chemical environment and, thus, function? Researchers have been examining this question for nearly a century. New evidence suggests that alcohol molecules do work directly on proteins in the membrane. Scientists think that LGICs contain protein binding pockets that can accommodate alcohol molecules.

Science does not yet have methods to detect if alcohol binds with these proposed protein binding pockets, because the bonds formed would be too weak. However, the scientists approached the problem another way. They knew that molecules of different types of alcohol contain different lengths of chains of carbon atoms, and that the longer the carbon chain, the more dampening effect an alcohol has on nerve cells -- up to a point. When the chain exceeds a certain length, alcohol's effect on the cells cuts off.® The scientists reasoned that cutoff occurs because longer carbon chains make alcohol molecules too big to fit into their protein pockets on the membrane. If this is so, they hypothesized, changing the structure of the protein pocket to be too small to receive an alcohol molecule with a long carbon chain also would result in cutoff.

*Advance:* Scientists created *in vitro* animal cells that contained human membrane proteins to which neurotransmitters usually bind to trigger ion-channel opening. By genetically altering the size of certain pockets in these protein structures, scientists changed the cutoff size for alcohol.

*Implications:* Many pharmaceuticals work by binding to specific proteins, altering their function. These ingenious experiments provide evidence of alcohol-binding sites on membrane proteins, providing potential targets for pharmaceuticals for alcoholism treatment. [secondary B treatment]

Wick MJ, et al.: Mutations of  $\gamma$ -aminobutyric Acid and Glycine Receptors Change Alcohol Cutoff: Evidence for an Alcohol Receptor? Proceedings of the National Academy of Science USA, 11(95):6504-6509, May 1998.

### **Alcohol Consumption Influenced by Different Genes in Females and Males, Suggesting that They May Process Alcohol Differently**

*Background:* A few years ago, scientists searching for the genes that contribute to alcoholism came up with a surprising result that they were not even pursuing, in addition to the information they sought. They found two genes that influenced alcohol consumption in mice. However, one of the genes had this effect only in male mice, while the other gene affected only female mice.

Scientists study alcohol disorders because they are common and costly. About 14 million adult Americans suffer from alcohol abuse or alcoholism. These disorders cost the Nation more than \$166 billion annually. Mice provide a valuable model for study, since the behaviors of mice that drink alcohol resemble those of heavy alcohol consumption in humans.

Researchers are studying mice to find which of their thousands of genes affect alcohol preference and alcohol avoidance. In the study described here, researchers used a statistical method that links traits to genes and gives the approximate location, on a chromosome, of the genes that contribute to a trait -- in this case, drinking. A trait is any physical or behavioral characteristic; for example, height, aggression, or preference for alcohol. In all living organisms, interactions of genes and environmental factors determine traits, including those associated with alcohol consumption. Several genes may affect a single trait, as is the case in alcoholism.

A major task facing scientists is to find the locations, on chromosomes, of the genes that govern specific traits. Scientists do mathematical analyses that indicate the likelihood that the genes for a given trait, chromosomal locations unknown, are near a gene whose chromosomal location is known. If both the trait whose genes=locations are unknown and the gene whose location already is known appear in offspring more often than would occur by chance, it is likely that the unknown genes are near the known gene on the chromosome. Scientists base this statistical method on the way chromosomes reshuffle when passing genes onto offspring in reproduction.

*Advance:* In previous studies, researchers found two genes, one on chromosome 2 and one on chromosome 11, that affect whether or not mice prefer alcohol. In this study, investigators identified two more, on chromosomes 3 and 1. All of the genes that have a major effect on alcohol preference in these breeds of mice appear to be gender-specific. Researchers also performed analyses to see if they had missed any genes that were major influencers of alcohol preference but were *not* gender-specific, and found none.

*Implications:* Recent studies suggest that genetic influence on human preference for alcohol may be gender-specific. This mouse study provides clues as to where scientists should look for gender-specific genes that affect alcoholism in humans. Genes are starting points for biochemical pathways that regulate behaviors. Identifying genes enables scientists to trace these pathways, looking for points for intervention. If different genes do affect alcoholism in men and women, identifying these genes could lead to gender-specific treatment and prevention methods. [secondary B treatment and prevention]

Pierce JL, Derr R, Shendure J, Kolata T, Silver LM: A Major Influence of Sex-Specific Loci on Alcohol Preference in C57Bl/6 and DBA/2 Inbred Mice. Mammalian Genome, 9:942-948, 1998.

### **Skeletal Muscle Damage Induces Heart Disease**

*Background:* Skeletal muscle constitutes 40-50 percent of body weight. Because of its extensive network of small blood vessels (microvasculature) and need for oxygenated blood, skeletal muscle is a major factor in the vascular system. Therefore, muscle damage and muscle disorders, such as Duchenne muscular dystrophy (DMD), present significant challenges to normal cardiac function. DMD is characterized by extensive and progressive damage to skeletal muscle. It results in immobilization and death in severely affected male youths. Frequently there is associated damage to the heart, or cardiomyopathy.

*Advance:* Using a newly developed strain of *mdx* mice lacking an important skeletal muscle determining factor, MyoD, investigators showed that progression of skeletal muscle damage is a significant factor leading to development of cardiomyopathy. In this animal model, muscle regeneration and healing are impaired because muscle satellite cells (a form of stem cells) do not follow a normal path of repair; rather, they remain in a proliferative, pre-muscle form. The mice develop a progressive cardiomyopathy, with areas of severe necrosis. Heart tissue has an increased level of stress-activated signaling components, and the left ventricle is significantly enlarged, indicating a response to increased resistance to blood flow due to muscle damage.

*Implications:* This new animal model provides opportunities to better understand the development of symptoms in DMD. The results indicate that disease becomes more severe when healing is impaired. The more severe skeletal muscle myopathy induces an increase in cardiac complications. Based on these findings, researchers should be able to design better interventions for the treatment of degenerative diseases, including DMD. Also, the findings indicate that it will be important to maintain muscle's healing capacity during any treatment in order to prevent further degeneration. [secondary B treatment]

Megeney LA, et al: Severe cardiomyopathy in mice lacking dystrophin and MyoD. Proc Natl Acad Sci USA 96: 220-25, 1999.

### **Tumor Necrosis Factor Mediates Orthopaedic Implant Osteolysis**

*Background:* Over 200,000 total hip replacements, which are initially successful, are performed each year in the United States for end-stage arthritis; eventually, however, a complication of total hip replacement can be osteolysis, the disappearance of bone around the implant in response to the presence of microscopic wear particles.

*Advance:* In a living mouse model, researchers demonstrated that tumor necrosis factor (TNF), a substance that modulates cell functions, is essential to development of wear particle-induced osteolysis.

*Implications:* This finding sets the stage for development of pharmacologic or biologic treatments that target TNF and thus prevent the disappearance of bone around orthopaedic implants. [secondary B treatment]

Merkel KD, et al.: Tumor necrosis factor alpha mediates orthopaedic implant osteolysis. Am J of Path 154: 203-10, 1999.

### **Molecular Basis of the Physical Connection Between Epidermis and Dermis**

*Background:* The physical connection between the epidermis and dermis (the two outermost living layers of skin) is mediated by a large number of molecules interacting in the basement membrane zone (the area between these two layers) of skin. Some of these molecules are within the basal cell layer of the epidermis, some are within the electron microscopic basement membrane zone, and others are in the upper dermis.

*Advance:* In a recent study, researchers used a molecular genetic approach to isolate a molecule implicated in blistering skin diseases. They proceeded to study how it interacts with other molecules and functions as an attachment molecule.

*Implications:* Understanding the interactions that underlie the physical connection between epidermis and dermis could lead to design of therapeutic interventions for autoimmune blistering skin diseases such as epidermolysis bullosa. [secondary B treatment]

Aho S, and Uitto J: 180-kD bullous pemphigoid antigen/type XVII collagen: Tissue-specific expression and molecular interactions with keratin 18. J Cell Biochem 72: 356-67, 1999.

### **Hair: Molecular Biology, Embryology, Cycling and Diseases**

*Background:* There has been a vast increase in scientific findings related to the normal structure and function of hair, its embryologic development, its cycling (moving between growing and resting phases), and defects in both genetic and acquired diseases. These have taken advantage of many advances in the molecular biology of the keratins (structural proteins of the hair), and somewhat unexpectedly, information concerning adhesion molecules in the skin (molecules that are involved in the attachment of the layers of skin to one another), as well as a variety of other basic research finds in both human biology and in mouse naturally occurring and transgenic (genetically altered) model systems.

*Advance:* Recent research reveals that a molecule that functions as an intracellular adhesion molecule, beta-catenin, also functions in a signaling process involved in the development of hair follicles. When this beta-catenin was inserted into epidermal skin cells, the genetically altered mouse skin in adult animals underwent a process resembling new hair development. The follicles, however, were disoriented and defective in certain signaling and polarization processes (which ensure normal hair cycling and hair shaft pointing in the right direction) , and proliferation continued unchecked, resulting in tumors that look like hair-follicle tumors seen in humans.

*Implications:* These findings indicate that it may be possible to initiate new hair formation in adult animals. [secondary B treatment]

Gat U, DasGupta R, Degenstein L, and Fuchs E: De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95: 605-14, 1998.

## **Growth Factors Prevent Loss of Embryonic Nerve Cells Exposed to Toxins in Test Tube**

*Background:* Fetal alcohol syndrome (FAS), the leading preventable birth defect in the United States, is caused by maternal drinking during pregnancy and results in mental retardation, learning disabilities, and physical defects. Most of these deficits persist throughout life. FAS costs U.S. society an estimated \$1.9 billion in 1992, for health care, special education, and lost productivity. This figure does not include related disabling alcohol-induced birth defects.

FAS researchers are focusing on how alcohol damages the nervous system of the fetus. The nervous system, which includes the brain and thousands of interacting nerve cells, guides every activity of the body, from movement to learning. Alcohol is known to have a direct toxic effect on fetal nerve cells and to create a uterine environment toxic to them. Other toxic conditions also are known to adversely affect the development of the fetal nervous system. For example, fetuses deprived of oxygen or glucose, the blood sugar that nourishes tissues throughout the body, may suffer loss of nerve cells. In addition to its direct toxic effects, alcohol can damage fetal nervous systems by creating an environment of oxygen deprivation and glucose deprivation.

In previous studies, researchers showed that certain substances can prevent nerve loss in animal fetuses exposed to toxic conditions. These substances are neurotrophic factors, naturally occurring brain proteins that promote growth of nerve cells. The researchers tested the effects of neurotrophic factors on nerve cells isolated from fetal animal brains and placed in incubation chambers, then exposed to alcohol alone or to oxygen deprivation or glucose deprivation alone. However, these conditions in isolation do not closely simulate the environment leading to FAS.

*Advance:* For the first time, researchers tested neurotrophic factors in embryonic rat nerve cells placed in incubation chambers and exposed to alcohol combined with glucose deprivation or oxygen deprivation. By testing these conditions together, researchers simulated more closely the environment of an embryo exposed to maternal drinking. They found that two neurotrophic factors, brain-derived neurotrophic factor and nerve-growth factor, but not glial cell line-derived neurotrophic factor, significantly prevented or ameliorated loss of nerve cells. The study also revealed that, while combining alcohol with oxygen deprivation or glucose deprivation moderately worsened outcome for fetal nerve cells, alcohol has the most potent toxic effects.

The researchers used cells of the hippocampus, an area of the brain that is especially vulnerable to alcohol damage and is involved in memory and cognition (knowing, awareness, and judgment). All of these abilities are vulnerable to impairment in FAS.

*Implications:* In an ideal world, preventing maternal drinking would be the solution to FAS. Research designed to prevent maternal drinking is ongoing. In the real world, however, preventing or reversing damage to fetuses exposed to alcohol is equally important. The highly protective effect of some neurotrophic factors against alcohol-induced embryonic nerve loss *in vitro* suggests potential therapeutic strategies for preventing FAS in the womb.[secondary -- prevention and treatment]

Mitchell JJ, Paiva M, Walker DW, Heaton MB. BDNF and NGF Afford *in vitro* Neuroprotection Against Ethanol Combined with Acute Ischemia and Chronic Hypoglycemia: Developmental Neuroscience, 21:68-75, 1999.

### **Violence Reduction Sustained After Alcoholics Receive Behavioral Marital Therapy**

*Background:* Each year, one in every six couples in the United States engages in an incident of physical assault. That heavy drinkers and alcoholics are more prone to engage in physical abuse toward their spouses is intuitive. Researchers not only have confirmed this conventional wisdom in scientific studies, but also have documented the extent to which alcohol-related domestic violence is a public-health problem. They found that more than 50 percent of alcoholics have abused a female partner in the year prior to alcoholism treatment.

Researchers are in the beginning stages of examining domestic violence among alcoholics. For example, scientists have found that alcoholism treatment based on behavioral marital therapy (BMT) significantly reduces risk of domestic violence. At first glance, this outcome might seem obvious. However, among the questions researchers are trying to answer, having established the immediate effectiveness of BMT in reducing spouse abuse, is whether or not this effect is lasting. Researchers also are studying other factors. For example, do spouse-abusing alcoholics who have stopped drinking continue to abuse their wives? Or is alcohol *per se* the driving force for domestic violence in this population?

In initial studies of a group of alcoholics undergoing BMT, researchers established a baseline and conducted a one-year follow-up. They found that, prior to BMT, these people were four to six times as likely to abuse their spouses and to abuse them more frequently than were members of a demographically similar comparison group of nonalcoholics. In the year after treatment, alcoholics who began drinking again were significantly more violent than the nonalcoholic comparison group. However, in the same post-treatment year, the alcoholics who remained abstinent from drinking were not more violent than their comparison-group counterparts. Researchers also found that, during this year, the number of days that a person drank correlated significantly with the frequency of his spouse abuse.

*Advance:* Researchers next conducted a two-year follow-up of the same group of alcoholics, described above, who underwent BMT. They found that outcomes were unchanged from those of the one-year follow-up; that is, in the second year follow-up,

- subjects engaged in significantly less domestic violence than they did in the year prior to BMT and
- abstinent alcoholics had domestic-violence levels similar to those of the nonalcoholic comparison group post-treatment, but alcoholics who began drinking again had elevated levels of domestic violence.

*Implications:* The study described here confirms and documents the powerful role of alcohol in spouse abuse -- although all of the alcoholics in the study underwent the same marital therapy, those who remained abstinent afterward had significantly lower levels of domestic violence than did those who resumed drinking. These findings suggest that treatments that are successful in sustaining abstinence are key to reducing domestic violence among alcoholics. [secondary B prevention and treatment]

O'Farrell TJ, Van Hutton V: Domestic Violence Before and After Alcoholism Treatment: A Two-Year Longitudinal Study. Journal of Studies on Alcohol, 60:317-321, 1999.

### **Light-to-Moderate Drinkers Account for More On-the-Job Problems than Do Heavy Drinkers**

*Background:* Work-related drinking can have serious consequences for employees and employers alike, in terms of injury and productivity. The problem affects a considerable portion of the workplace population. For example, in a recent study of a major, Midwestern, Fortune-500 assembly plant with a traditional organizational structure, 25 percent of workers reported engaging in on-the-job drinking. Of these, 43 percent usually had two or more drinks, and 19 percent reported drinking on the job at least once a month.

Recently, the Robert Wood Johnson Foundation and the NIH jointly funded a study designed to answer questions about how different drinking behaviors affect job performance. For example, alcohol is known to affect numerous physical and cognitive functions, such as awareness and judgment, at the time of consumption. But does heavy drinking the night before work have measurable, significant effects on job performance the next day, even though blood-alcohol level has returned to zero? Does chronic off-the-job drinking affect job performance? How do these scenarios compare to job performance of employees who drink at work? What implications do the answers to these questions have for employers?

To study these issues, researchers surveyed 6,540 workers, managers, and supervisors from seven large corporations that represented a variety of organizational settings and company cultures. The researchers focused on four variables: (1) average daily volume of alcohol, indicating level of drinking, (2) drinking on the job, (3) dependence on alcohol, and (4) frequency of drinking to get drunk.

*Advance:* Not surprisingly, researchers found that drinkers in the moderate-to-heavy and heavy categories reported more work-performance problems than did very light, light, or moderate drinkers. However, people in the three categories of lighter drinkers were more numerous than those in the heavier-drinking categories. Consequently, the lighter-drinking group actually accounted for a larger proportion of work-performance problems than did the heavier-drinking group.

Both drinking on the job and heavy drinking during off-work hours negatively affected job performance. Drinking on the job, dependence on alcohol, and frequency of drinking to get drunk were significantly associated with poorer work performance, even after researchers took into account other factors that might have had an impact, such as negative life circumstances and job characteristics.

*Implications:* These findings suggest that employers should not focus exclusively on efforts to reach alcohol-dependent workers, but should expand their efforts to inform all employees about relationships between drinking and work performance. The findings also indicate the need for policies that clearly limit drinking while on the job. [secondary B prevention and treatment]

Mangione TW, Howland J, Amick B, Cote J, Lee M, Bell N, Levine S: Employee Drinking Practices and Work Performance. *Journal of Studies on Alcohol*, 60:261-270, 1999.

## **One In Four U.S. Children Exposed to Alcohol Abuse or Alcoholism in Family**

*Background:* The effects of alcoholism extend far beyond alcoholics themselves. For example, children in families affected by alcohol often live in environments that are stressful, chaotic, and frightening. Frequently, they are neglected or abused and face economic hardship and social isolation. Children of alcoholics are vulnerable to mental illness and medical problems, and are more likely than others to become alcoholic at some point in life.

In 1992, the NIH conducted the largest national survey on alcohol use ever performed in the U.S. or elsewhere. This research revealed that almost 14 million U.S. adults meet medical criteria for a diagnosis of alcohol abuse (such as binge drinking) or alcoholism. Given the magnitude of alcohol disorders among U.S. adults and the harm these adults can impose on children, epidemiologists sought to determine how many U.S. residents age 17 or younger are exposed to alcoholism or alcohol abuse via a member of the family.

*Finding:* Using 1992 survey data, epidemiologists recently estimated that almost 43 percent (28,046,258) of U.S. children lived in households with one or more adults who had been alcoholics or alcohol abusers at some point in life. Approximately 15 percent of these children (9,667,463) lived in households with an adult diagnosed in the past year. Most of the children (82.5 percent) were biological, foster, step-, or adopted children of these adults.

Epidemiologists considered other factors in their final estimate. For example, they assumed that only half of the children living with an adult diagnosed with an alcohol-use disorder prior to the past year might suffer adverse consequences. Using these and other criteria, epidemiologists calculated that one in four U.S. children is exposed to alcohol abuse and alcoholism in the family. This figure probably is conservative, since it does not include homeless children.

*Implications:* Children at risk for consequences of exposure to familial alcoholism constitute a major public health problem. Currently, social and health services for these children are fragmented and often do not address the far-reaching effects of familial alcohol exposure. The findings described here illustrate the urgent need to establish a comprehensive strategy for children at risk that will integrate existing services, broaden them, and target each developmental stage of childhood. [secondary B prevention]

Grant, BF. Children Exposed to Alcohol Abuse and Dependence. [American Journal of Public Health](#), in press.

## Newly Discovered Genes May Contribute to Epilepsy

*Background:* Electrical storms in the brain underlie the seizures of epilepsy. In nerve cells electrical charge is carried by ions, like the positively charged sodium ion and negatively charged chloride ion of sodium chloride, table salt. Ions flow in and out of nerve cells through tiny pores, called ion channels, that cross the cells' outer membrane. Ion channels control the flow of ions like electrical switches by rapidly opening and closing their pores. Not surprisingly, several types of inherited epilepsy are caused by defects in ion channels that lead to uncontrolled electrical activity in the brain. In 1998 scientists discovered defects in two genes for potassium ion channels that cause familial neonatal epilepsy, but which of the many types of potassium channels those genes specified was not clear.

*Advance:* This year scientists learned that the two genes that are affected in familial neonatal epilepsy code for subunits that together make up a ion channel called the M channel that allows potassium ions to enter and leave cells. The M channel has been intensively studied since the early 1980s, but the gene for this channel was not known. From those studies we know that the M channel is an important regulator of electrical activity in nerve cells. The M channel is important because opening of the channel is regulated by both the voltage across the cell membrane and by certain neurotransmitters, the chemical signals by which nerve cells communicate.

*Implications:* This finding solves two mysteries—what causes a form of epilepsy and which gene codes for the M channel. Bringing together a clinical problem and a long-standing focus of research in the fundamental electrical behavior of the brain reveals a key target for developing more specific drugs to treat epilepsy. [secondary B treatment]

Wang H-S et al.: KCNQ2 and KCNQ3 Potassium Channel Subunits: Molecular Correlates of the M-Channel. Science 282:1890-1893, 1998.

Barinaga M: Steadying Influence for Neuron Identified. Science 282:1794-1795, 1998.

Leppert M, Singh N: Benign Familial Neonatal Epilepsy with Mutations in Two Potassium Channel Genes. Curr Opin Neurol (2):143-147, 1999.

Singh NA et al.: A Novel Potassium Channel Gene, KCNQ2, is Mutated in an Inherited Epilepsy of Newborns. Nature Genetics 18:25-29 1998.

Charlier C et al.: A Pore Mutation in a Novel KQT-like Potassium Channel Gene in an Idiopathic Epilepsy Family. Nature Genetics 18:53-55, 1998.

## **Does Moderate Alcohol Intake Protect the Heart? Scientists Track Pathways that Could Lead to Cardioprotection**

*Background:* To scientists, biochemical pathways can be like puzzles. These complex pathways are series of molecular interactions that end in predictable physiological outcomes. Scientists identify the various steps in biochemical pathways -- like finding the pieces of a puzzle -- to help them establish a picture of the mechanisms that underlie physical phenomena. If the pathway is part of a process that leads to disease, scientists look for points at which to block the pathway at the molecular level, with pharmaceuticals.

Pathways also can be part of beneficial processes. For example, many studies have suggested that moderate alcohol consumption may have a protective effect on the heart, and scientists are studying the pathways that could lead to this effect. One area of study involves a phenomenon called *Aporeconditioning*.<sup>®</sup> It has to do with ischemia (deprivation of blood flow and, thus, oxygen to body tissues, resulting in damage to them). In a seeming paradox, previous animal studies demonstrated that brief periods of ischemia can protect the heart muscle in certain circumstances. These brief ischemic periods of the heart, while transiently damaging, reduce or prevent death of heart-muscle cells if the heart later undergoes the kind of sustained ischemia that usually would result in a heart attack. Moderate, prolonged alcohol intake appears to have this preconditioning effect. The pathways that lead to preconditioning are typically complex. Among the pieces of the puzzle is the enzyme (a protein that initiates chemical reactions) protein kinase C (PKC).

Scientists have tested the protective effects that prolonged, moderate alcohol exposure appears to confer against major ischemia. In the study described below, researchers instead tested the effects of brief alcohol exposure just before major ischemia. They also developed and tested a substance that blocks a PKC variant in the pathway that results in alcohol's protective effect, to determine if the protection occurred even without the PKC variant.

*Advance:* Researchers found that, in incubated heart-muscle cells from rats, brief alcohol exposure just before major ischemia protected the cells from damage. Blocking PKC inhibited alcohol's ability to protect the cells.

*Implications:* Do these findings mean that the public should be advised to drink moderate amounts of alcohol? The suggested benefits of alcohol must be weighed against its many known risks. Alcohol plays a role in several types of health problems, including some types of cancer, liver disease, and injuries resulting from drunk driving. Not the least among alcohol-induced health problems is risk for alcoholism, from which 14 million adult Americans suffer and which costs the Nation \$166 billion annually.

Ultimately, researchers seek to isolate substances that may be key elements in cardioprotection. If further research confirms these substances' protective effect, researchers will seek to design methods of administering them as pharmaceuticals, without the risks that accompany alcohol consumption. [secondary B prevention]

Chen CH, Gray MO, Mochly-Rosen D. *Cardioprotection from Ischemia by a Brief Exposure to Physiological Levels of Ethanol: Role of Epsilon Protein Kinase C*. Proceedings of the National Academy of Science, in press.

### **Steroid-Induced Bone Loss: Mouse Findings Point to Preventive Possibilities**

*Background:* For thousands of transplant patients, glucocorticoids, a class of steroidal immunosuppressive medications, are life-sustaining: the drugs keep their bodies from rejecting the newly transplanted organs. Thousands more patients also use these drugs for chronic diseases as rheumatoid arthritis, lupus and asthma. But despite their apparent success, glucocorticoids have a substantial shortcoming: long-term users often develop osteoporosis, serious bone weakening and loss that puts the body at risk for fractures and subsequent disability. Scientists have long been interested in learning the mechanism behind glucocorticoid-induced bone loss, reasoning that the mechanism held the key to improved treatment and prevention of this loss.

*Advance:* Investigators have used a mouse model and cell culture techniques not only to shed new light on glucocorticoids' destructive mechanism, but to point the way to preventive measures. The team of scientists has shown that mice treated with prednisolone, a commonly used glucocorticoid, have bone loss similar to that seen in human patients. The research team also found that the prednisolone-treated mice had reduced numbers of bone-forming osteoblasts; glucocorticoid reduced the rate of osteoblast formation in cell cultures of mouse bone-marrow cells; and a greater number of dying bone cells were present in prednisolone-treated mice than in untreated mice. These scientists discovered that the dying bone cells included not only osteoblasts, which are present on bone surfaces, but osteocytes, which are imbedded in the bone itself and are thought to help stimulate bone formation. This finding paralleled an increase in dying osteoblasts and osteocytes in patients with glucocorticoid-induced bone loss.

*Implications:* This study shows that glucocorticoids may both reduce the formation of new bone cells and increase the death of cells important to new bone cell development. Programmed cell death occurs at increased rates in bone cells during glucocorticoid treatment. If glucocorticoids act as a signal to osteoblasts and osteocytes to begin the dying process, and if the nature of that signal can be determined, it may be possible to design drugs to block the signal and spare many bone cells that might otherwise be lost. For thousands of transplant patients, arthritis and asthma sufferers, and others at risk for steroid-induced bone loss, that's good news. [secondary B prevention]

Weinstein, et al. Inhibition of Osteoblastogenesis and Promotion of Apoptosis of Osteoblasts and Osteocytes by Glucocorticoids. *J Clin Invest* 102:274-282, 1998.

### **Risk of Hip and Wrist Fractures Shown Linked to Chromosome 19 Gene**

*Background:* Osteoporosis, a major threat for 28 million Americans, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures—especially of the hip, spine, and wrist. It is the most prevalent of the bone diseases that affect Americans. Osteoporosis is known to have a genetic component, but genes associated with fractures themselves had not been found before.

*Advance:* Osteoporotic hip and wrist fractures—a common feature on the landscape of older Americans—may be partially rooted in a gene on chromosome 19, according to a study funded by the NIH (co-funded by NHLBI and NIA). Scientists have found that older women with the gene for apolipoprotein E (APOE\*4) are at increased risk for hip and wrist fractures. Previous studies of this gene have also shown its association with common, late-onset forms of Alzheimer's disease and with osteoporosis in patients on dialysis. The study showed that the risk of hip and wrist fracture for women age 65 and over with the APOE\*4 gene was nearly twice that of those without the gene, even after making adjustments for bone density, cognitive level, or tendency to fall. Women with at least one APOE\*4 gene were more likely to have a maternal history of fracture after age 50.

*Implications:* Scientists have long suspected that these types of fractures are due to more than a single factor, and this finding provides evidence of a specific genetic influence at work even when weak bones and balance problems are not at issue.

The APOE\*4 gene might involve the following factors in increasing risk for hip and wrist fractures: (1) Vitamin K. People with the gene may have reduced levels of this substance, which stimulates bone formation and reduces bone-cell loss; (2) Alzheimer's disease. People with this disease have a higher risk of hip fracture, and the APOE\*4 gene has now been found to have connections to both; (3) Weight loss. Women with the gene experience greater weight loss than those who don't have it. Weight loss contributes to bone loss, which could affect fracture risk. [secondary B prevention]

Cauley JA, Zmuda JM, Yaffe K, Kuller LH, Ferrell RE, Wisniewski SR, Cummings SR. Apolipoprotein E polymorphism: a new genetic marker of hip fracture risk—the study of osteoporotic fractures. *Journal of Bone and Mineral Research* 1999;14(7):1175-1181.

### **Physical Activity and Osteoarthritis**

*Background:* Known as a wear and tear disease, osteoarthritis (OA) is associated with physically demanding activity. Knowing whether physical force or excess loading initiates OA or acts as an agent of progression on an injured or congenitally misaligned joint would help physicians frame exercise recommendations.

*Advance:* Researchers using participants in the Framingham Study found that individuals who engaged in more than 4 hours of heavy physical activity per day were 7 times more likely (13 times, if obese) to develop knee OA than individuals who did no heavy physical activity. Fortunately, walking and light physical activities, usually recommended for older individuals, did not contribute to increased risk of OA.

*Implications:* The finding suggests that excess force on otherwise normal joints contributes to the cartilage degradation characteristic of osteoarthritis and could lead to better exercise recommendations for those suffering from OA. [secondary B prevention]

McAlindon TE, Wilson PWF, Aliabadi P, Weissman B, and Felson DT: Level of physical activity and the risk of radiographic and symptomatic knee osteoarthritis in the elderly: The Framingham Study, Am J Med 106: 151-57, 1999.

### **Restoring Production of Brain Cells in Old Age**

*Background:* The hippocampus is a brain area that plays an important role in learning and memory, especially the episodic memory for personal experiences that is impaired in many elderly individuals. Nerve cells in one part of the hippocampus, called the dentate gyrus, are unusual in that they are continually replaced; that is, some cells die and others are born, even in adults. In aging humans and rats, the brain is exposed to high levels of corticosteroids, a type of steroid hormone, from both prolonged stress-induced secretion and increased base-line levels. These adrenal stress hormones are suspected to cause or accelerate damage of the hippocampus, leading to memory impairment associated with normal aging.

*Advance:* Scientists have now shown that reducing corticosteroid hormone levels in aged rats restores the rate of nerve cell proliferation in the dentate gyrus of the hippocampus. Scientists reduced hormone levels in the rats by removing the adrenal glands which produce corticosteroids, but less drastic reductions are also likely to have an effect. The findings suggest that the hippocampus of even old rats can generate new nerve cells as well as in young rats, but that in aging this process is slowed by high levels of corticosteroids.

*Implications:* This work suggests new insights into age-related memory loss and new avenues for the development of therapies to prevent memory loss in aging. [secondary prevention]

Cameron HA and McKay RD: Restoring production of hippocampal neurons in old age. [Nature Neuroscience](#) in press.

### **Killing of Intracellular *Mycobacterium tuberculosis* by an Antimicrobial Protein Found in Human Cytotoxic T Lymphocytes**

*Background:* *Mycobacterium tuberculosis*, the cause of tuberculosis (TB), is also the leading cause of bacterial infection and death, worldwide. Combating *M. tuberculosis* presents a challenge due to its ability to thrive both outside and inside host cells. Within cells are spherical capsules called vesicles. It is within the vesicles of infected cells that *M. tuberculosis* is shielded from antibodies and many other defensive molecules, contributing to its prolonged persistence in the host. However, an important group of cytotoxic T lymphocytes (CTL), termed CD8+ CTL, can act upon hidden tubercle bacilli. These CTL attack cells that Aflag® their infection by displaying fragments of bacterial antigens on their surfaces. CD8+ CTL destroy the infected cells, causing release of cellular contents. However, disruption of host cells does not by itself eliminate infection. In fact, lysis B the rupture of the infected cell B by other types of CTL releases viable *M. tuberculosis* that can infect new cells. The pathway by which CD8+ CTL destroys *M. tuberculosis* was not known.

*Advance:* Investigators sought to identify the molecules used by CD8+ CTL to eliminate *M. tuberculosis*. Scientists discovered that, during an attack on infected cells, CD8+ CTL release a bacteria-killing protein, called granulysin, plus a pore-forming protein, called perforin. The perforin molecules open holes in the membranes of the target cells, enabling granulysin to attack the internal bacteria. Granulysin directly disrupts *M. tuberculosis* within the cells, terminating the intracellular infection.

*Implications:* This research has defined a specific pathway by which certain immune CTL kill complex intracellular pathogens. Development of strategies to preferentially induce CTL that utilize the granulysin pathway is a very promising avenue for new vaccine research and development to control TB. Because granulysin has antimicrobial activity against a wide-range of bacteria, fungi, and parasites, the ability to induce strong CD8+ CTL by vaccination may be applicable to a variety of intracellular infections. [secondary B prevention]

Strenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich C J, Ganz T, Thoma-Uszynski S, Melián A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, & Modlin RL: An antimicrobial activity of cytolytic T cells mediated by granulysin. Science 282:121-124, 1998.

### **Ancient Receptors Trigger Inborn Reactions to Bacteria**

*Background:* Septic shock caused by bacterial infection results in thousands of deaths annually. Septic shock is a severe reaction to bacterial cell wall components, chief among which are lipopolysaccharides (LPS), large molecules made up of specific lipids (fats) and saccharides (sugars). The discovery, over thirty years ago, of an LPS-unresponsive mouse strain, called C3H/HeJ, clarified that the biological activity of LPS is due to genetically-controlled host responses rather than to direct toxic properties of bacterial cell walls themselves. A long search for the cellular proteins that mediate responsiveness to LPS has led to the discovery of a family of receptors, molecules that detect and signal the presence of bacteria cell walls. These receptors appear to function not only in septic shock, but also in providing important signals that activate protective immune responses to bacterial infection and vaccination.

*Advance:* The link between LPS recognition and the activation of cellular responses was shown to be a group of evolutionarily-conserved proteins, termed Toll-like receptors, that play a role in host defense in such distantly-related organisms as mammals and fruit flies. Investigators observed that the expression of one Toll-like molecule, human Toll-like receptor 2 (hTLR2), was increased on certain cells in the presence of LPS. The researchers showed that hTLR-2 functioned along with two co-receptors, designated LPS-binding protein and CD14, to signal the presence of LPS. A similar result was obtained by genetic analysis of the defective chromosomal region of the C3H/HeJ mouse, leading to the isolation of the murine Toll-like receptor 4 (mTLR4) protein, which is molecularly similar to the hTLR2 protein receptor in humans.

*Implications:* Knowledge of the structure and function of Toll and other protein receptors may facilitate development of new vaccine strategies and new approaches to the treatment of septic shock. Drugs that could interfere with the activation of Toll-like receptors by bacteria during an acute infection could save thousands of lives by blocking the septic shock signaling cascade. Toll-like receptors may also provide new avenues for the rational design of vaccine adjuvants, which are mixtures of biologically active materials -- such as bacterial cell walls -- that boost immune responses to antigens. Scientists may be able to construct molecules that retain the ability of LPS to trigger Toll-like receptors, but lack some of the harmful properties of whole bacterial cell walls. The design of new adjuvants that elicit stronger, longer lasting immune responses, with reduced toxicity, would be a major advance in vaccine science. [secondary B treatment]

Hoffmann JA, Kafatos FC, Janeway Jr., CA & Ezekowitz RAB: Phylogenetic perspectives in innate immunity. *Science* 284: 1313-1318, 1999.

### **Vigorous Cytotoxic Response Leads Recovery against Hepatitis C Virus**

*Background:* Hepatitis C virus (HCV) is a major cause of chronic liver disease, cirrhosis, and liver cancer worldwide. Although the majority of HCV-infected individuals develop virus-specific antibody and cellular immune responses (such as generation of protective cytotoxic lymphocytes called CTL), more than 70 percent of exposed individuals go on to develop chronic hepatitis C. The failure to eradicate HCV in these cases is probably due to multiple factors. However, a major factor is that HCV undergoes frequent mutation (genetic change) during the course of infection. Each mutation slightly alters the fundamental structure of each pathogen. This results in the accumulation of a variety of distinct forms of the same pathogen within an infected individual. The immune system, which was able to recognize the pathogen in its original form, has difficulty recognizing the new variants. This ability of HCV to exist in multiple forms in one host allows HCV to evade host immune defenses. Although humans are the natural host for HCV, it is difficult to study patients in the early stages of HCV infection. The only other animal species in which HCV infection becomes chronic is the chimpanzee.

*Advance:* Six chimpanzees were inoculated with HCV to study their immune responses to infection. Chimpanzees that recovered without becoming chronically infected were found to generate strong CTL responses in the liver. This response was characterized by the early appearance of CTLs that recognize a wide range of components of HCV proteins. These CTLs can suppress or eliminate HCV in the liver and may destroy HCV-infected cells. In contrast, the study found no correlation between antibody production and HCV infection. Although this does not eliminate a role for antibodies in the control of HCV, a broad and vigorous CTL response appears to be a more important factor in the early resolution of disease.

*Implications:* This insight into the ability of a broad, organ-localized, CTL response to clear infection may contribute to the development of new, more potent, immunotherapies and to vaccine design. Moreover, this insight may have implications for better understanding other viral pathogens, such as HIV, that mutate frequently and thus exist in multiple forms in infected hosts. [secondary B treatment]

Cooper S, Erickson A, Adams A, Kansopon J, Weiner A, Chien D, Houghton M & Walker C: Analysis of a successful immune response against Hepatitis C virus. Immunity 10:439-449, 1999.

### **The Mammalian Gene Collection: A Resource for Studying Gene Expression and Function**

*Background:* With the imminent completion of the DNA sequence of the human genome, the challenge remains to identify the functional units, genes, and determine their role in health and disease. To facilitate these studies, scientists need a critical research tool, a catalog of the full repertoire of human genes. In early FY1999, several NIH Institutes launched a coordinated effort to develop these tools.

The genetic instruction packets in DNA are the genes. To carry out their functions within cells, gene are first copied into messenger RNA (mRNA) molecules that are in turn used as templates for production of all the proteins necessary for biological processes. In the 1970=s molecular biologists figured out ways to copy mRNA molecules in a test tube to make replicas (cDNAs) that were amenable to study. To examine the function and interaction of thousands of genes, researchers need a resource of full-length cDNAs reflecting all the genes in the genome as well as the computational and informatics tools for their analysis.

*Advance:* In FY1999, the NIH launched the Full-length Mammalian cDNA Initiative. The project=s scientific goals are to produce cDNA clone collections that contain full-length copies of all genes, sequence theses cDNAs, develop the associated informatics tools, and create a publicly accessible website to provide up-to-date information to the research community.

A Request for Applications was issued to solicit research proposals for improving technology for full-length cDNA production and analysis. Grants will be awarded by the end of FY1999. An NIH working group (comprised of both extramural and intramural scientists) is overseeing the generation of full-length cDNA clones. Production cDNA sequencing will be supported by contracts to be awarded in FY2000. An External Advisory Committee, comprised of scientists from academia and industry, will be convened early in FY2000 to oversee and guide the project.

*Implications:* The generation and availability of clones and sequences of the complete set of human genes and those of other mammals will be a critical research tool enabling researchers to readily explore the function of genes and further our understanding of biology and human health.

Strausberg RL, Feingold EA, Klausner RD., Collins FS: The Mammalian Gene Collection. Science (in press, 1999).

## Genetic Defect in Myeloid Leukemia Explained

*Background:* Cancer is a genetic disease. Leukemia is among the deadliest of cancers. In the United States, 11,000 new cases of myeloid leukemia, one of the two main categories of leukemia, are diagnosed each year. Acute myeloid leukemia (AML) occurs in all age groups and has a cure rate of only 25%. Subtypes of AML are diagnosed by the appearance of the blood cells that proliferate without control, and each subtype now appears to be caused by different genetic defects.

*Advance:* One particular type of AML is caused by a rearrangement on one chromosome, human chromosome 16, that flips a large chunk of DNA and brings together two usually separate genes. The abnormal, new *Afusion* gene that's created codes for a fusion protein that joins a muscle structural protein with a protein that normally regulates the development of blood cells. Scientists recently discovered why creating this fusion gene is so destructive. Directed by its muscle protein attachment, the regulatory protein winds up in parts of the cell far from where it needs to be to work. Instead of hitching up with partner regulatory proteins to direct the orderly formation of blood cells, the fusion protein winds up coating structural filaments in the cell. Mice that have this fusion gene also develop leukemia, but the mouse model shows that at least one more, unknown mutation is required before full-fledged AML arises.

*Implications:* Scientists are now working to find what mutations in mice B and people B are required in addition to the fusion gene to cause AML. Understanding the genetic basis for AML should lead to medical treatments that improve the cure rate. As it is, one subtype of AML, caused by a rearrangement between human chromosomes 15 and 17, has a better cure rate than the others because scientists understand what gene is disrupted in this subclass of leukemia and have learned how to get around the genetic disruption with drugs. [secondary B treatment]

Castilla LH, Garrett L, Adya N, Orlic D, Dutra A, Anderson S, Owens J, Eckhaus M, Bodine D, and Liu PP: Chromosome 16 inversion-generated fusion gene *Cbfb-MYH11* blocks myeloid differentiation and predisposes mice to acute myelomonocytic leukemia. Nature Genetics (in press for October), 1999.

Adya N, Stacy T, Speck NA, and Liu PP: The leukemic protein CBFb-SMMHC sequesters CBFa2 into cytoskeletal filaments and aggregates. Mol Cell Biol 18:7432-43, 1998.

### **Absence of Linkage between Bone Formation and Bone Loss**

*Background:* In healthy adults, bone mass is maintained at a constant level through a balance between bone formation and bone resorption or loss. The apparent linkage between these two processes led scientists to presume that there were regulatory signals exchanged between cells responsible for these two antagonistic yet complementary processes. However, the precise linkage mechanism has never been demonstrated. An implication of this model of bone mass maintenance is that a defect in the cells responsible for either bone formation or loss should lead to a decrease in function of the other cell type in order to maintain a constant bone mass.

*Advance:* An international team of scientists has succeeded in producing a new mouse model with a genetic defect in the cells that are responsible for bone formation (osteoblasts). To accomplish this work, a gene was inserted into mouse eggs linked to genes which are active in bone forming cells. The inserted gene made the bone-forming cells susceptible to an antibiotic, so that these cells could be specifically killed. The antibiotic was administered to young mice when most, but not all, of their skeletal growth had occurred. The resulting loss of osteoblasts not only arrested skeletal growth, but there was also evidence that bone resorption, measured as a dramatic decrease in bone mass, was unaffected by the loss of the bone-forming cells.

*Implications:* These advances support the concept that bone formation and resorption are not reciprocally linked, but appear to be separately regulated. In addition, the mouse model used in these studies appears to be a new tool to study bone loss conditions such as osteoporosis.

Corral DA, Amling M, Priemel M, Loyer E, Fuchs S, Ducy P, Baron R, and Karsenty G: Dissociation between bone resorption and bone formation in osteopenic transgenic mice. Proc Natl Acad Sci USA 95: 13835-40, 1998.

### **Mechanism of Fungal Adhesion Identified**

*Background:* *Candida albicans*, a fungal microorganism, is one of the many microbes that normally inhabit the human body. It usually exists at low levels in the oral cavity and gastrointestinal tract. However, when the immune system is compromised, as is the case for HIV/AIDS patients and patients undergoing cancer treatments, *Candida albicans* can grow to higher levels and result in opportunistic infections such as oral candidiasis or thrush. Painful, creamy white patches appear in the mouth and esophagus, and can impair swallowing. Some candidal infections can be relatively mild. On the other hand, these fungal infections are among the most common nosocomial or hospital-acquired infections and have the potential to become life threatening blood-borne systemic infections. Oral candidiasis is the most common oral fungal infection in HIV-infected patients, and is found in about 45 percent of AIDS patients. *Candida albicans* is a yeast that usually grows through a budding process. In immune-suppressed patients, this organism grows by sending out hyphae or filaments that spread across and penetrate into the epithelial cells that line the oral cavity. These filaments adhere tightly to the cells of the mucosal epithelium and promote further growth.

*Advance:* A recent NIH-funded study focused on the mechanism of filament attachment or adhesion. Earlier work had demonstrated that *Candida* hyphae contained a hypha-specific surface protein, Hwp1, that bound to a common mammalian enzyme, transglutaminase, and identified the gene responsible for production of that protein - HWP1. The role of Hwp1 in attachment was explored by developing strains of *Candida* with and without the HWP1 gene. Strains lacking Hwp1 were found unable to form stable attachments to human buccal epithelial cells and also were shown to have a reduced ability to cause systemic candidiasis in a mouse model.

*Implications:* Hwp1 appears to be an important factor in the invasive tight attachment of *Candida albicans* to oral epithelial cells. By understanding the mechanism of adhesion, it becomes possible to find ways to disrupt the initiation of pathology. Future studies can now focus on finding ways to inhibit adhesion. Ultimately, identification of adhesion inhibitors should point the way to treatments that effectively interfere with the colonization, growth and proliferation of this opportunistic yeast in immune-compromised patients. [secondary B treatment]

Staab JF, Bradway SD, Fidel PL, and Sundstrom, P: Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. Science 283: 1535-38, 1999.

## Why Prostate Cancer Homes to Bone

*Background:* Prostate cancer is the second leading cause of cancer deaths in men in both Europe and the United States. At the time of diagnosis, about 40 percent of the cases have metastasized outside the prostate gland. From there, some forms of prostate cancer show a high propensity for invading bone in the pelvis and vertebra of the lower back. One of the mysteries surrounding prostate cancer has been its seeming predilection for bone. Is this because other tissues are not amenable to its advances or does bone send out some sort of homing signal that draws the metastasizing cells? The question has been why bone rather than nearby organs like kidney, liver, or even lung. One theory has been coined "seed and soil," which likens cancer cells to seeds drifting in the wind and landing at random on different types of soil--putting down roots only where the local conditions allow. Another theory portrays cancer more like a predatory animal that is drawn by the trail of its favorite prey.

*Advance:* NIH intramural scientists led an international research team to determine if there was a specific factor in bone that acts as an attractant for prostate and possibly other bone-seeking cancers, such as breast cancer. The investigators studied the migration of prostate cancer cells to bone, brain, liver and kidney tissue extracts. The bone tissue extract attracted four times as many prostate cancer cells. Further analysis of the bone tissue extracts found a single component responsible for the attraction B the protein osteonectin, which is present primarily in bone and thought to be involved in the mineralization process. The attractive ability of bone tissue extracts was eliminated by pre-treatment with an antibody that binds specifically to osteonectin. Additional tests showed that osteonectin also increases the invasiveness of prostate cancer cells by increasing production of enzymes that promote tissue invasion. Osteonectin also stimulated enzyme activity in breast cancer cells, another form of cancer that readily metastasizes to bone.

*Implications:* By studying the reaction of prostate cancer cells to various solutions of tissue extract, scientists found that a bone protein caused the cells to aggressively migrate to the bone extract. Although this work is still in the laboratory stage, the investigators suggest that a compound, such as an antibody that inhibits osteonectin, may act against bone-seeking cancers by discouraging the spread of the cells to bone and also by preventing the production of invasive enzymes. Such an approach may provide leads for future treatments to inhibit the bone metastases that frequently arise from such common neoplastic diseases as prostate and breast cancer. [secondary B treatment]

Jacob K, Webber MM, Benayahu D, and Kleinman HK: Osteonectin promotes prostate cancer cell migration and invasion: A possible mechanism for metastasis to bone. Cancer Research in press, 1999.

## Major New Tumor Suppressor Provides Fresh Insights into Cancer

*Background:* Tumor suppressor proteins provide crucial defenses against cancer. Gene mutations crippling these proteins allow cancer cells to survive and form potentially lethal tumors. A new tumor suppressor gene, PTEN (MMAc), was discovered in 1997 and found to be mutated in significant percentages of a variety of human cancers, including metastatic cancers of the prostate, malignant melanomas, brain tumors called glioblastomas, breast, lung, head-and-neck, and other cancers. Studies have shown that PTEN is an enzyme that removes phosphate from regulatory lipids and proteins involved in signaling processes. Restoring PTEN to tumor cells with defective PTEN blocks their growth.

*Advance:* PTEN functions in both tumor cell invasion and survival. Malignant cells have altered internal signaling and external interactions that lead to abnormal cell migration and tissue invasion. PTEN inhibits such migration and invasion by acting on two central signaling molecules, FAK and Shc. These molecules play pivotal roles in controlling internal cell signaling, cell migration, and other interactions mediated by receptors called integrins. The second target of this dual-function protein is an important signaling lipid that controls the process of programmed cell death (apoptosis). Cells that lose contact with their normal environmental cues normally stop multiplying, and most die by apoptosis. Malignant cells can bypass this protection system. Restoring PTEN to brain tumor cells results in more normal signaling and increased cell death (apoptosis) after loss of cell contacts. Dual sites of action on key cellular signaling systems may contribute to the function of PTEN as a tumor suppressor. There are now several mouse models with defective PTEN genes mimicking aspects of human cancer.

*Implications:* PTEN is known to target central regulators of cell behavior governing adhesion, migration, invasion, growth, and programmed cell death. These findings are beginning to define the exact blend of cell signaling processes needed for developing or suppressing cancer. An important factor in eliminating cancer cells is determination of how a cell in the wrong location can be triggered to die, thus preventing uncontrolled growth, tumor formation, and metastasis. PTEN and the molecules it regulates provide one such mechanism. PTEN could be restored to tumor cells by gene therapy, and detailed knowledge of how it functions could also provide novel targets for drugs that mimic PTEN actions to suppress cancer progression. [secondary B treatment]

Tamura M, Gu J, Takino T, and Yamada KM: Tumor suppressor PTEN inhibition of cell invasion, migration, and growth: Differential involvement of focal adhesion kinase and p130<sup>Cas</sup>. Cancer Res 59: 442-49, 1999.

Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE, and Parsons R: Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci USA 96: 1563-68, 1999.

Tamura M, Gu J, Danen EHJ, Takino T, Miyamoto S, and Yamada KM: PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. J Biol Chem 274: 20693-703, 1999.

Gu J, Tamura M, Pankov R, Danen EHJ, Takino T, Matsumoto K, and Yamada KM: Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. J Cell Biol 146: 389-404, 1999.

### **Secretory Leukocyte Protease Inhibitor (SLPI) Inhibits Arthritis**

*Background:* Injury and infection elicit a complex series of reactions in the host designed to isolate and/or eliminate the foreign agent as well as to minimize and repair tissue damage. Precise regulation of these mechanisms is crucial for the maintenance of tissue integrity, and malfunction may result in tissue destruction characteristic of rheumatoid arthritis and other chronic inflammatory diseases. Disruption of the balance between enzymes known as proteases and protease inhibitors is often associated with pathologic tissue destruction.

*Advance:* To explore the therapeutic potential of secretory leukocyte protease inhibitor (SLPI), an inhibitor of enzymes made by neutrophils and other cells, in erosive joint diseases, NIH intramural investigators cloned, sequenced and expressed active rodent SLPI which shares the enzyme-reactive site with human SLPI. In a rat model of inflammatory erosive arthritis, injection of SLPI inhibited joint inflammation and destruction of cartilage and bone. Inflammatory pathways as determined by blood levels of tumor necrosis factor and activation of cellular transcription factors were inhibited by SLPI. Additionally, SLPI was able to suppress collagenase cleavage of type II collagen which is found in cartilage and which can be measured by the presence of cleavage products in the blood.

*Implications:* These data indicate that the actions of SLPI may extend beyond the inhibition of serine proteases to functions which are important to resolution of inflammation. [secondary B treatment]

Song X, Zeng L, Jin W, Thompson J, Mizel DE, Lei K, Billingham RC, Poole AR, and Wahl SM: Secretory leukocyte protease inhibitor suppresses the inflammation and joint damage of bacterial cell wall-induced arthritis. Journal of Experimental Medicine 190: 535-42, 1999.

### **Acceleration of Wound Healing in Aged Humans**

*Background:* Wound healing problems in the elderly impose a substantial burden on health services, affecting over 4 million people annually and costing in excess of 9 billion dollars. Delays in acute cutaneous wound healing lead to local infection, separation of wound layers (dehiscence) and a shift toward a chronic nonhealing wound. Age and hormones appear to be of great importance in modulating acute wound repair, both in terms of the rate of healing and the degree of scarring incurred. Specifically, aging is associated with reduced levels of a protein which promotes cell migration and formation of scar tissue (TGF-beta), increased wound neutrophil numbers and neutrophil elastase activity leading to excessive tissue breakdown and a delayed monocyte influx.

*Advance:* New studies by NIH intramural scientists and international collaborators have shown that the hormone estrogen, administered both topically and systemically, can reverse age-related changes in acute human wound healing and in animal models of aging. The acceleration of healing was associated with reduced neutrophil numbers, reduced elastase activity and decreased tissue breakdown, but with increased monocyte infiltration and TGF-beta levels. *In vitro* mechanistic studies suggest that estrogen has direct effects on neutrophil migration and expression of adhesion molecules.

*Implications:* These data demonstrate that delays in wound healing in severe diabetes and in the elderly can be significantly diminished by topical estrogen in both males and females. This may prove to be a novel and cost-effective treatment. [secondary B treatment]

Ashcroft GS, Greenwell-Wild T, Horan MA, Wahl SM, and Ferguson MWJ: Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. *American Journal of Pathology* 155: in press, 1999.

### **Breast Cancer, Heart Disease, Osteoporosis and the $\beta$ ERKO Mouse**

*Background:* Observational data indicates that too much, or too little, of the hormone estrogen is implicated in the development of a variety of diseases. These diseases include breast cancer, endometriosis, uterine fibroids, osteoporosis, and heart disease. The exact role that estrogen plays in setting the stage for these diseases, however, is unknown. Estrogen itself is a potent regulator of many cellular events in men and women. The initiating step for all estrogenic activity is the binding of estrogen to specific protein receptors in the cell. Understanding the role of these receptors in initiating estrogen-controlled processes is a vital first step to defining how estrogen acts to maintain health.

*Advance:* Rodent models provide one of the most useful ways to study significant biological events in a timely and reproducible manner. These same models can be genetically modified to further enhance their use. One such technique is to eliminate or knock-out the function of the gene coding for a particular protein and then to study biological events in the knock-out mice and see how they compare with unaltered (wild type) animals. A few years ago, an estrogen receptor knock-out (ERKO) mouse was constructed for the estrogen receptor known as estrogen receptor  $\alpha$ , or ER $\alpha$ . This estrogen receptor is broadly distributed throughout the body. What has been missing is a knock-out of the second, or  $\beta$ , estrogen receptor, which, though less widely distributed than ER $\alpha$ , is found at high levels in the ovary, prostate, epididymis, lung, and hypothalamus. This year NIH-supported scientists were able, in collaboration with an international group, to develop the  $\beta$ ERKO mouse, which lacks functional estrogen receptor  $\beta$  (ER $\beta$ ).

Male and female  $\beta$ ERKO mice show no apparent abnormalities and are able to breed, although litter sizes are smaller than average due to reduced ovarian efficiency. This contrasts with the marked anomalies found in the  $\alpha$ ERKO mice, such as an absence of breast development in females and infertility caused by reproductive tract and gonadal and behavioral abnormalities in both sexes.

*Implications:* This model, along with the  $\alpha$ ERKO mouse, provides a means by which scientists can assess the role of estrogen in a broad array of diseases, including cancers of the breast, ovaries, and uterus, endometriosis, uterine fibroids, osteoporosis, and heart disease. These models can also be used to determine if environmental compounds can interfere with estrogen receptor-mediated events, and thus affect health. Such compounds would include phytoestrogens such as those found in soy products, which are thought to reduce risk of breast and prostate cancers, and organochlorine insecticides, which are suspected of increasing breast cancer risks.

This model also provides insight into the normal function of estrogen receptors and indicates ways in which mutations to these receptors can affect human health. For example, the fact that ovarian function is suboptimal in  $\beta$ ERKO mice, even though they are normal appearing, suggests that some fertility problems in women could be due to variations, or polymorphisms, in the gene coding for ER $\beta$ , which could lead to reduced ovarian efficiency. These possibilities can be pursued in a clinical setting.

Krege JH, et al. (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor  $\beta$ . Proc. Nat. Acad. Sci. USA 95:15677-15682.

### **A Common Link in Failed Pregnancies**

*Background:* The road to birth is a perilous one. NIH scientists showed in 1988 that 25 percent of fertilized eggs fail to survive six weeks— a point so early in pregnancy that most women did not know they had been pregnant. Only daily urine testing for shifting traces of a hormone called human chorionic gonadotrophin (hCG) confirmed these pregnancies and their loss. The 25 percent early loss, when added to the clinical miscarriages that occur later, means that at least one-third of all embryos fail. Now, this same group has shown that embryos that implant in the uterus late are more likely to die in the first weeks of pregnancy.

*Advance:* Implantation of the fertilized egg into the wall of the uterus is a critical step in fetal development. In a study in which daily urine samples were collected from about 200 North Carolina women, researchers found that the lost fetuses tended to be those that implanted late. For those women having full-term pregnancies, the fetuses had implanted only about one day earlier, on average, than the non-survivors: 9.1 days vs. 10.5 days from fertilization to implantation. But the day-by-day trend was clear. If a fertilized egg implanted by the ninth day, it had only a 13 percent chance of loss. The risk rose to 26 percent if the implantation was on the tenth day, 52 percent on the eleventh day, and 82 percent thereafter. There was no association in this study between late implantation and miscarriages that occurred later in pregnancy.

Additional research will be needed to determine why embryos implant late. It is possible that embryos that implant more slowly may be imperfect in some way. Thus, the uterus may be receptive to pregnancy only during a limited time-window, shutting out defective embryos that get there too late. This would spare a mother the physiologic burden of supporting a non-viable embryo.

*Implication:* This study shows that early pregnancy loss takes place within in a very small time-window and that this is the time frame investigators need to focus on in future studies. Clearly events surrounding fertilization and implantation of an egg represent a highly vulnerable time and one in which any environmental disturbance could have devastating effects. A better understanding of the critical implantation events and of how environmental disturbances can delay these events will lead to more successful strategies to ensure future pregnancies.

Wilcox A, Baird DD, and Weinberg CR (1999) Time of implantation of the conceptus and loss of pregnancy. New Engl. J. Med. 340:1796-1799.

### **Breast Cancer Susceptibility Gene, BRCA1 - How Does It Work?**

*Background:* In 1996, the first breast cancer susceptibility gene, BRCA1, was discovered. Mutations to this gene account for 40 percent to 50 percent of inherited breast cancers and also confer an increased risk of ovarian and prostatic cancers. What remains to be discovered is the normal biological role of BRCA1 and how mutational damage to BRCA1 acts to increase cancer risk.

*Advance:* Stimulation of breast tissue by the hormone, estrogen, can lead to increased cell growth and turnover of mammary epithelia. This event could be a factor in promoting development of breast cancer. A group of NIH-supported scientists examined the ability of BRCA1 protein to regulate cellular responses to estrogen by conducting experiments using estrogen receptor  $\alpha$  (ER $\alpha$ ) assays. ER $\alpha$  is the protein to which estrogen binds. It is this estrogen-ER $\alpha$  binding that initiates the cascade of molecular signaling events that are regulated by estrogen, including increased growth of breast tissue.

Using cultured cells from human prostate cancer, human breast cancer, and human cervical cancer lines, these researchers studied the activity of BRCA1 both in the presence and absence of ER $\alpha$ . In these transient transfection assays, the wild type, or unmutated, BRCA1 was found to inhibit signaling by the activated estrogen receptor. This finding raises the possibility that BRCA1 suppresses estrogen-dependent tissue growth and that, when damaged, this control is lost and excessive tissue growth, or tumorigenesis, can occur. Interestingly, only the prostate cancer and breast cancer cell lines were responsive to BRCA1. The cervical cancer cells were not responsive, a finding verified by the fact that BRCA1 does not seem to be implicated in increased risk to cervical cancer.

*Implications:* Understanding the biological role of BRCA1 enhances our ability to derive intervention strategies for treatment of breast cancers, prostate cancers, and ovarian cancers that arise from mutated forms of BRCA1.

Fan S, Wang J-A, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auburn KJ, Goldberg ID, Rosen EM (1999) BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science* 284:1354-1355.

### **Inhibitors of Growth Factors Inhibit Pulmonary Fibrosis**

*Background:* The diseases caused by inhaled inorganic particles, such as asbestos and silica, have been with us for centuries, but we are just now beginning to understand the basic molecular mechanisms involved. Chronic asbestosis and silicosis share a fundamental disease mechanism with other respiratory responses to environmental insult: pulmonary fibrosis. Pulmonary fibrosis is a disease of inflammation that results in scarring, or fibrosis, of the lungs. In time, this fibrosis can build up to the point where the lungs are unable to provide oxygen to the tissues of the body.

Pulmonary fibrosis may be initiated by a variety of factors, including chemotherapeutic drugs such as bleomycin and metals such as cadmium and vanadium. The disease is characterized by the excessive proliferation of mesenchymal cells (fibroblasts, myofibroblasts, and smooth muscle cells) and the deposition of extracellular matrix proteins, especially collagen, by these cells.

*Advance:* NIH researchers, using a rat model of vanadium-induced lung fibrosis, have found that specific inhibitors that block phosphorylation of tyrosines on platelet-derived growth factor receptor proteins or epidermal growth factor receptor proteins can inhibit the growth of epithelial and mesenchymal cells within the fibrotic lesions. This inhibition results in a reduction of collagen deposition in the lung.

*Impact:* Although great strides have been made in protecting workers from the adverse effects of exposures to respirable particles, both occupational and non-occupational cases of pulmonary fibrosis continue to occur, constituting a significant public health problem. Research on these mechanisms of lung injury and their modulation by drugs can provide leads for treatment strategies. The findings contained in this report provide strong evidence that these particular receptors are important to the progression of pulmonary fibrosis and suggest that targeting autophosphorylation of receptor tyrosine kinases could have potential therapeutic value in treating fibroproliferative lung disease. [secondary B treatment]

Rice AB, Moomaw CR, Morgan DL, and Bonner JC: Specific inhibitors of platelet-derived growth factor or epidermal growth factor receptor tyrosine kinase reduce pulmonary fibrosis in rats. American Journal of Pathology 155: 213-221, 1999.

### **Subtle Mutations Can Have Disastrous Effects When Combined**

*Background:* Single polymorphisms—small differences in genetic makeup between individuals—occur throughout the human genome. In some cases, they may contribute to some individuals' propensity towards development of some diseases as well as to differences in responses to environmental conditions and exposures. In addition, combinations of some subtle mutations for which there are individually few negative consequences might turn out to have deleterious effects when combined. Because genetic instability—changes in or damage to the DNA in the cell—is a major causative factor in the formation of tumors, the consequences of subtle functional alterations in proteins that act on DNA are of particular interest. Such combinations would be difficult to identify in traditional genetic linkage studies. An alternative approach is to use experimental organisms in which interactions between polymorphisms of particular genes can be easily generated and studied.

*Advance:* Researchers working with yeast cells created strains with several combinations of mutations affecting DNA metabolic proteins. Looking specifically at an endonuclease called Rad27—a protein whose function is to chop up DNA at specific sites—they selectively inactivated the nuclease activity as well as the nuclease's binding site for another protein called proliferating cell nuclear antigen (PCNA), which normally stimulates the Rad27 nuclease activity when it binds. The mutation eliminating PCNA binding had very little effect by itself on mutation, recombination, and sensitivity of the cells to the DNA-damaging agent methyl methanesulfonate (MMS), but when combined with a mutation in a DNA repair gene, it greatly increased the MMS sensitivity ordinarily conferred by the DNA repair mutation (up to 1,000-fold).

*Impact:* These results suggest that natural, well-tolerated single polymorphisms might have disastrous consequences when combined, which would have implications for understanding and treating genetically based diseases, for differential responses to environmental stress, and for distinguishing and compensating for drug efficacy and tolerance. Studies in model systems can help to direct epidemiological studies to specific pairs of genes (such as the endonuclease and DNA polymerase genes) where severe interactions between otherwise neutral polymorphisms are most likely to occur.

Gary R, Park MS, Nolan JP, Cornelius HL, Kozyreva OG, Tran HT, Lobachev KS, Resnick MA, and Gordenin DA: A novel role in DNA metabolism for the binding of Fen1/Rad27 to PCNA and implications for genetic risk. Molecular and Cellular Biology 19: 5373-5382, 1999.

## Signaling Environmental Stress B How Do Cells Respond to Their Surroundings?

*Background:* Cells in different organs of the human body process and integrate a huge amount of information about their local environment through a complex array of protein messengers. These messengers transduce signals from the outside of the cell across the cell membrane, through the cytoplasm and ultimately into the cell nucleus. In acute times of stress, either environmental stress such as ultraviolet or ionizing radiation, or endogenously generated pro-inflammatory stress in response to infection or cell injury, cells undergo a precise and rapid response to the particular stressor agent in its environment. Movement or stimulation of specific proteins, called transcriptional activators, into the cell nucleus lead to alterations in gene expression in response to environmental stress. These changes in gene expression lead to the formation of new proteins that help the cell respond to the potentially harmful conditions. One such condition inside the body occurs during infection or cell injury in which specific cells induce cytokines which trigger an inflammatory response. Chronic stress associated with inflammatory response can lead to wide number of diseases including heart disease, autoimmunity, asthma, arthritis, neuronal degradation, and cancer.

*Advance:* In a series of seminal studies these researchers have followed the precise molecular events which occur inside the cell to activate the expression of new genes in response to specific stress signals like those mentioned above. It was found that the activation of a specific transcription factor, NF- $\kappa$ B, by the degradation of an inhibitory molecule, I- $\kappa$ B, involves both phosphorylation events and protein degradation events. These researchers have engineered specific mice deficient in this degradation pathway, specifically by knocking out protein which phosphorylates I- $\kappa$ B, in order to dissect the specific steps in this complex cascade of events. Surprisingly, these mice were capable of mounting a specific NF- $\kappa$ B stress response, but had specific alterations in the development of their limbs, skeleton and skin.

*Implications:* In order to better treat a broad range of diseases associated with pro-inflammatory signals (heart disease, asthma, autoimmune diseases, and others as mentioned above), it is essential to understand the underlying molecular biology of the stress responses. These basic cutting-edge studies provide specific molecular targets for the development of novel intervention strategies in the treatment of these diseases. [secondary B treatment]

Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, Karin M The IKK $\beta$  subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. J. Exp. Med. 189:1839-1845, 1999.

Baud V, Liu ZG, Bennett B, Suzuki N, Xia Y, Karin M Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes. Dev. 13:1297-1308, 1999.

Delhase M, Hayakawa M, Chen Y, Karin M. Positive and negative regulation of IkappaB kinase activity through IKK $\beta$  subunit phosphorylation. Science 284:309-313, 1999

Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, Johnson R, Karin M: Abnormal morphogenesis but intact IKK activation in mice lacking the IKK $\alpha$  subunit of IkappaB kinase. Science 284: 316-20, 1999

## Chemokines and Multiple Sclerosis

*Background:* Multiple sclerosis is a leading cause of neurological disability among young adults. The disease is characterized by the loss of myelin, the fatty electrical insulation that wraps nerve fibers and enables rapid and reliable electrical signaling. What causes the loss of myelin is not clear, but inflammation and infiltration and direct attack by immune cells of the brain and spinal cord have been implicated. Decades of research in immunology have revealed that immune cells, in general, enter tissue and cause inflammation in response to specific chemical signals, including a large family of molecules called chemokines that activate specific receptors, that is detectors, on the immune cells.

*Advance:* A research team has now identified specific chemokines called IP-10, Mig, and RANTES--that are consistently elevated in patients undergoing attacks of multiple sclerosis. Furthermore, the immune cells implicated in inflammation during this disease carry the specific receptors that detect and respond these chemokine signals.

*Implications:* These chemokines and their receptors present targets for the development of drugs to directly and specifically prevent the inflammation that causes damage in multiple sclerosis. The results also provide important insights into how the disease progresses by clarifying the chemical signals that provoke immune cells to cross the blood brain barrier, which normally excludes them, and cause damaging inflammation within the brain and spinal cord. Similar processes are likely to play an important role in other inflammatory disorders that affect the brain and spinal cord. [secondary B treatment]

Sereneness T, Tania M, Jensen J, Pierce V, Lucchinetti C, Folcik V, Qin S, Rottman J, Sellebjerg F, Strieter R, Frederiksen J, Ransohoff R: Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. Journal of Clinical Investigation 103:807-815, 1999.

## SCIENCE CAPSULES

**Anchoring Fibrils.** Anchoring fibrils, which consist of a specific type of protein known as collagen type VII, attach layers of skin (dermis and epidermis) to each other. Investigators focusing on the molecular mechanisms by which collagen type VII anchors the dermis to the epidermis have discovered that interference with its binding to a specific molecule in the basement membrane zone of the skin, laminin 5, may underlie the skin blistering that occurs in both hereditary and acquired epidermolysis bullosa. Identification of the genetic defect at the molecular level can enable genetic testing and counseling and disease severity prediction.

Chen M, Marinkovich MP, Jones JC, O'Toole EA, Li YY, and Woodley DT: NC1 domain of type VII collagen binds to the beta 3 chain of laminin 5 via a unique subdomain within the fibronectin-like repeats. J Invest Dermatol 112: 177-83, 1999.

**Lupus Diagnosis and Survival.** The Rochester Epidemiologic Project (REP) is a unique resource in the United States that allows identification and retrieval of inpatient and outpatient medical records of residents of Olmsted County, Minnesota 94 percent of whom are Caucasian. In a new, population-based study of systemic lupus erythematosus (SLE) incidence and mortality data covering a 40-year period, REP investigators reported that the incidence had nearly tripled and there was a statistically significant improvement in the survival trend. These data provide the most current information on the frequency and impact of SLE in a well-defined community.

Uramoto KM, Michet CJ, Thumboo J, Sunku J, O'Fallon WM, and Gabriel SE: Trends in the incidence and mortality of systemic lupus erythematosus, 1950-1992. Arth & Rheum 42: 46-50, 1999.

**Genetic Studies of Systemic Lupus Erythematosus (SLE).** Researchers have found an association between SLE and a region on chromosome 1. Fine mapping of this region has identified another candidate gene involved in immune function, specifically in the processes of DNA repair and cell death both of which have been reported to be abnormal in SLE. Studies of the genetics of SLE allow identification of at-risk individuals prior to development of disease, potential therapeutic strategies targeting the involved genes or gene products, and the possibility of developing markers of disease activity and/or response to treatment.

Tsao et al.: PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. J Clin Invest, in press.

**Friedreich's Ataxia.** Friedreich's ataxia is a progressive disease that attacks the nervous system, the heart, and the pancreas. In 1996 an international group of scientists discovered the gene that, when defective, causes the disease, but the function of *frataxin*, the protein produced by that gene, was unknown. By examining a similar protein in yeast cells, scientists found a possible role in regulating iron metabolism. Now, a new magnetic resonance technique for brain imaging has shown that iron levels are indeed selectively elevated in the brains of patients with this

disorder, suggesting that focusing on iron may provide a possible avenue for the development of treatment.

Waldvogel D, van Gelderen P, Hallett, M: Increased iron in the dentate nucleus of patients with Friedreich's ataxia. Annals of Neurology 46:123-5, 1999.

**The Brain's Capacity to Change.** In the last several years scientists have learned that the human brain can change to a surprising degree in response to experience, which is called brain plasticity. One of the most dramatic examples of brain plasticity is that people who are blind use the area of the brain normally devoted to processing vision when they read Braille. Recent studies show that this rewiring of the brain is possible only through the teenage years. Understanding the extent and limitations of the brain's ability to change is one of the most important avenues for finding ways to help patients recover from brain damage from disease or injury.

Sadato NA, Pascual-Leone J, Grafman, Deiber MP, Ibanez V, and Hallett M: Neural networks for Braille reading in the blind. Brain 121:1213-29, 1998.

Cohen LG, Weeks RA, Sadato N, Celnik P, Ishii K, Hallett M: Period of susceptibility for cross-modal plasticity in the blind. Annals of Neurology 45:451-60, 1999.

**A New Approach to Recovery After Spinal Cord Injury.** Many long nerve fibers are normally coated with an electrical insulator called myelin, which enables nerves to rapidly and reliably conduct movement control signals and sensory information. In about half of patients with spinal cord injury some nerve fibers are spared, but their myelin insulation is lost or abnormal, reducing their ability to carry sensory and movement signals. New studies based on understanding of the genes that control myelin formation suggest that unspecialized cells are present in the injured spinal cord that might be coaxed to become myelin forming cells if the appropriate control signals can be identified. If so, this will offer an important treatment strategy for treating spinal cord injuries. Even small increases in the number of normally functioning nerve fibers can substantially increase the quality of life following spinal cord injury.

Wrathal JR, Li W, and Hudson LD: Myelin gene expression after contusive spinal cord injury. J Neuroscience 18: 8780-8793, 1998.

**Imaging Pain in Humans.** Treating prolonged, intractable pain is one of the most important and daunting challenges that confronts health care professionals today. Despite recent advances in understanding the mechanisms of pain, millions of people continue to suffer, and we know relatively little about how higher brain centers produce the subjective experience of pain. Until recently doctors could only rely on what patients communicated to gauge the severity of pain and to understand how the brain processes pain signals. Now, for the first time, scientists using functional brain imaging techniques such as MRI and PET have begun to map the activity of specific brain areas while people actually experience acute and chronic pain. While it may take

years to fully unravel the implications of this new information, brain imaging studies, hopefully, will lead to new approaches for treatment and prevention of pain.

Apkarian AV, Darbar A, Krauss BR, Gelnar PA, and Szeverenyi NM: Differentiating cortical areas related to pain perception from stimulus identification: temporal analysis of fMRI activity. *J Neurophysiol* 81(6): 2956-63, 1999.

**Structural Basis of Multidrug Recognition Determined.** Resistance to antibiotics is an ever-increasing problem. One mechanism through which bacteria protect themselves from the effects of antibiotics is the expulsion of various toxins using membrane proteins known as multidrug-efflux transporters. Antibiotics bind to these proteins and the whole complex is transported out of the cell. Recently, scientists have determined the structure of the protein that serves as a key regulator of the synthesis of these proteins. This study provides a promising model for understanding the principles of drug resistance and may offer a clue of a method to overcome drug resistance by controlling the synthesis of key components in the resistance machinery. [secondary B treatment]

Zheleznova EE, Markham PN, Neyfakh AA, and Brennan RG: Structural basis of multidrug recognition by BmrR, a transcription activator of a multidrug transporter. *Cell* 96: 353-362, 1999.

**Structure of Key Component in Programmed Cell Death Determined.** Inadequately regulated programmed cell death can lead to cancer, immunodeficiency, infertility, and neurodegenerative disorders. Researchers have determined the three-dimensional structure and they are investigating the function of one representative member of a family of proteins that are key regulators of programmed cell death. This will provide insights into how this subset of molecules mediates cell death and survival.

Chou JJ, Honglin L, Salvesen GS, Yuan J, and Wagner G: Solution structure of BID, an intracellular amplifier of apoptotic signaling. *Cell* 96: 615-624, 1999.

McDonnell JM, Fushman D, Milliman CL, Korsmeyer SJ, and Cowburn D: Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. *Cell* 96: 625-634, 1999.

**Understanding Clathrin Mediated Endocytosis.** Endocytosis, the uptake of extracellular material into cells that is crucial for an array of cellular functions, can occur by several mechanisms. One such mechanism is mediated by the formation of clathrin cages, assembled from several clathrin proteins, which transport membrane cargo around the cell and break apart after successful transport to form new cages elsewhere. Recent determination of the three-dimensional structure of several proteins that help make up the clathrin cage are leading to a better understanding of the relationship between structure and function in endocytosis mediated by clathrin.

Musacchio A, Smith CJ, Roseman AM, Harrison SC, Kirchhausen T, and Pearse BMF: Functional organization of clathrin in coats: combining electron cryomicroscopy and X-ray crystallography. *Molecular Cell* 3: 761-770, 1999.

Ybe JA, Brodsky FM, Hofmann K, Lin K, Liu SH, Chen L, Earnest TN, Fletterick RJ, and Hwang PK: Clathrin self-assembly is mediated by a tandemly repeated superhelix. Nature 399: 371-375, 1999.

**New Explanation for Why Lactic Acid in Bloodstream Rises During Severe Injury, Sepsis, and Heart Failure.** Following severe injury, sepsis, or early heart failure, patients often have high blood levels of lactic acid that are traditionally thought to be a reflection of inferior blood flow to tissues. Recent studies suggest that the high levels of lactic acid are actually the result of elevated levels of epinephrine and epinephrine-like substances, in these patients. These new findings suggest that the current clinical strategy of increasing cardiac output and blood flow, may be ineffective and perhaps even harmful to patients by unnecessarily stressing the heart and kidneys. [secondary B treatment]

James JH, Wagner KR, Leffler RE, Upputuri RK, Balasubramaniam A, Friend LA, Shelly DA, Paul RJ, and Fischer JE: Stimulation of both aerobic glycolysis and  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity in skeletal muscle by epinephrine or amylin. American Journal of Physiology 277: 176, 1999.

Luchette FA, Robinson BRH, Friend LA, McCarter F, Frame SB, and James JH: Adrenergic antagonists reduce lactic acidosis in response to hemorrhagic shock. Journal of Trauma 46: 873-880, 1999.

**Crystal Structure of Enzyme That Produces Key Cell Molecule Determined.** Nitric oxide is a chemical messenger that participates in diverse cellular processes, including the regulation of blood pressure, neurotransmission, and the immune system's response to infection. Nitric oxide is produced by a family of enzymes collectively called nitric oxide synthases (NOS). Researchers have deciphered the three-dimensional shape of one form of NOS. This finding provides the foundation for a better understanding of the workings of this molecule and for the development of agents that will selectively stimulate or inhibit NOS in specific target tissues. [secondary B treatment]

Raman CS, Li H, Martasek P, Kral V, Masters BS, and Poulos TL: Crystal structure of constitutive endothelial nitric oxide synthase: a paradigm for pterin function involving a novel metal center. Cell 95: 939-50, 1998.

**Molecular Basis of Spinal Muscular Atrophy Discovered.** Basic researchers have discovered the molecular basis of spinal muscular atrophy (SMA), a neuromuscular disease characterized by degeneration of motor neurons leading to progressive limb and trunk paralysis and the most common genetic cause of infant mortality. Patients with SMA have reduced levels or mutations in the Survival of Motor Neurons (SMN) protein. Researchers have now found that SMN plays a crucial role in the production of messenger RNA. This work on the function of the SMN protein is expected to open up the search for therapeutics. [secondary B treatment]

Pellizzoni L, Kataoka N, Charroux B, and Dreyfuss G: A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell 95: 615-624, 1998.

**Broader Role of Telomerase in Extending Cell Life.** Normally, with each cycle of cell division, the ends of the chromosomes (telomeres) become shortened, until the chromosomes become unstable and the cells stop dividing: cancers develop when cells increasingly ignore, or override, such signals for chromosome stability. Telomerase, an enzyme, is capable of maintaining the telomeres, thereby extending the life span of a cell. Basic researchers have determined that telomerase also can lengthen the life of some connective tissue cells indefinitely by a second mechanism, capping the ends of the telomeres regardless of telomere length so they do not shorten and cause the chromosomes to become unstable. Efforts are underway to manipulate the enzyme for therapeutic purposes, whether to prompt cell division in order to replenish stocks of healthy cells, as is needed by bone marrow transplant recipients, or to interrupt the excessive cell proliferation that occurs in cancer cells. [secondary B treatment]

Zhu J, Wang H, Bishop JM, and Blackburn EH: Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. Cell 96: 3723-3728, 1999

**Induction of Tolerance to Antigens.** The process in which white blood cell lymphocytes (T-cells) react to one's own normal tissue as antigens (called an autoimmune reaction) is common to several types of disease, but is of special importance in type 1 diabetes. The current study examined the onset of T-cell tolerance (that is, non-reactivity) to self in the insulin-secreting beta cells of the pancreas. Investigators used a mouse (a transgenic mouse) given the genetic material necessary for expressing influenza virus hemagglutinin (HA) in pancreatic beta cells to study tolerance induction. The researchers found that in the newborn period the HA-specific T-cells were not tolerant--infection with influenza virus led to specific destruction of the beta cells in the pancreas leading to the development of autoimmune type 1 diabetes. Over time, the ability to induce autoimmune destruction gradually decreased until the adult mice were tolerant to the influenza virus infection and were protected from developing diabetes. This work provides evidence for the developmental regulation of tolerance induction in the cells of the body. The regulation of tolerance induction is particularly important in type 1 diabetes and these results may further explain the vulnerability to type 1 diabetes in the juvenile period. [secondary B prevention]

Morgan DJ et al., *Ontogeny of T Cell Tolerance to Peripherally Expressed Antigens*. Proc. Nat. Acad. Sci. 1999; 96:3854-8.

**Genetic Determination of Type 1 Diabetes Autoimmunity.** This prospective study was undertaken to assess the relative contributions of genetics and environment in determining onset of reactivity against one's own insulin secreting tissues (islet cell autoimmunity). The results showed that non-diabetic identical twin siblings of patients with type 1 diabetes had a significantly higher risk of progression to diabetes than non-identical twin siblings. At the last follow-up, 41.5% of the identical twin siblings expressed such reactivity (developed autoantibodies) for type 1 diabetes, compared with 20% of non-identical twin siblings, 10.7% of non-twin siblings, and 5.9% of controls. Twelve of 53 non-index identical twin siblings developed diabetes during follow-up while none of the 30 non-identical twins developed

diabetes. Mean follow-up was 12.2 years for the identical twin siblings and 5.9 years for the non-identical twin siblings. The investigators concluded that identical and non-identical twins differ in progression to diabetes and expression of islet-cell autoantibodies, with non-identical twin siblings similar to non-twin siblings. These observations suggest that genetic factors play an important part in the development of islet cell autoimmunity. [secondary B treatment and prevention]

Redondo MJ; Rewers M; Yu L; Garg S; Pilcher CC; Elliott RB; Eisenbarth GS, *Genetic determination of islet cell autoimmunity in monozygotic twin, dizygotic twin, and non-twin siblings of patients with type 1 diabetes: prospective twin study.* Brit. Med. J. 1999; 318:698-702.

**Intensive Therapy of Type 1 Diabetes Reduces Collagen-Based Complications.** Products of abnormal sugar metabolism (AAAdvanced glycation end products®, abbreviated AGE) and sugar-containing (glycated) proteins accumulate in tissues in diabetic patients and are thought to play a role in the development of blood vessel complications. Studying patients who participated in the large multi-institutional clinical trial known as the Diabetes Control and Complications Trial, or DCCT, offers an opportunity to assess skin collagen abnormalities in a large group of patients with type 1 diabetes who had been well characterized with regard to their blood sugar (glycemic) control and the status of eye, kidney, and nerve complications over the preceding five-to-six year period. Analysis of skin biopsies taken from DCCT patients showed that five years of intensive treatment (when compared to conventional treatment) was associated with a reduction in glycated collagen products. Further analysis indicated that glycated collagen was the parameter most consistently associated with diabetic complications -- even more so than hemoglobin marker (known as HbA1c) of blood sugar level. Continued monitoring of these patients may determine whether glycation products in the skin have the potential to provide new and improved tools to predict the future risk of developing complications. [secondary B prevention]

Monnier VM; Bautista O; Kenny D; Sell DR; Fogarty J; Dahms W; Cleary PA; Lachin J; Genuth S, *Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications.* Diabetes 1999; 48:870-80. (DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial.)

**Prediction of Coronary Disease in Type 1 Diabetes.** The reason for the development of an excess cardiovascular risk in type 1 diabetes is not well-explained. The presence of low-density (LDL) cholesterol increases risk but is only weakly predictive, and its concentration is often normal in type 1 diabetes. This study utilized data from a prior epidemiological study to determine whether antibodies to oxidized LDL (AB-OxLDL) and LDL-containing immune complexes, rather than LDL concentration, are predictive of coronary artery disease (CAD) in type 1 diabetes. The results showed that antibodies to oxidized LDL were a significant independent predictor (along with previously recognized predictors, such as hypertension) of CAD in type 1 diabetes. Increased oxidation of many proteins and of LDL in particular have been described in patients with diabetes. The researchers concluded that oxidation of LDL and

the immune response it elicits may play a role in the pathogenesis of CAD in type 1 diabetes and explain at least some of the enhanced CAD risk seen in type 1 diabetes. [secondary B prevention]

Orchard TJ; Virella G; Forrest KY; Evans RW; Becker DJ; Lopes-Virella MF, *Antibodies to oxidized LDL predict coronary artery disease in type 1 diabetes: a nested case-control study from the Pittsburgh Epidemiology of Diabetes Complications Study.* Diabetes 1999 Jul;48(7):1454-8.

**A Mathematical Model of Colonization by H. pylori, Pathogen in the Stomach.** A mathematical model of the equilibrium (symbiotic relationship) between H. pylori and the human host was developed, based on experimental evidence. The model included the host response to infection, and tested variations in both the bacterium and the host: strain variation, competition between strains for adherence sites (often resulting in a heterogeneous population of strains), immunodeficiency in the host, and others, including nutrients and possible mechanisms of the effects observed. The model is capable of allowing testing of new treatments quickly and inexpensively without the need for human clinical trials. It can improve our understanding of H. pylori colonization, and appears to be generalizable to other persistent microbial states, such as malaria, tuberculosis, and HIV/AIDS. [secondary B treatment]

Blaser MJ and Kirschner D, *Dynamics of Helicobacter pylori Colonization in Relation to the Host Response.* Proc. Natl. Acad. Sci USA 1999; 96:8359-64.

**Cholesterol-rich regions in membranes are key for Stress Effects on Blood vessel lining cells.** The cells that line blood vessels (endothelial cells) are susceptible to acute and chronic shearing forces as blood flows through the vessels. Shear stress selectively and differentially regulates activation (expression) of many genes that are important in diseases of blood vessel wall function. This is accomplished through the stress-induced production of various chemical factors that are known to regulate gene expression. It is through these responses that shear stress controls vascular tone, vessel wall remodeling, binding of blood cells to the vessel wall, and general balance of the blood system. This study showed that it is the cholesterol-rich membrane areas of endothelial cells that respond to shear stress by activating some of these chemical signaling factors. Future research to uncover additional signaling domains within the cell membrane will lead to a greater understanding of vessel wall injury, such as in atherosclerosis, and to ways of preventing and treating such injuries. [secondary B prevention]

Park Heonyong, Go Young-Mi, St. John Patricia, Maland Matthew C, Lisanti Michael P, Abrahamson Dale R, Jo Hanjoong, *Plasma membrane cholesterol is a key molecule in shear stress-dependent activation of extracellular signal-regulated kinase.* J Biol Chem 1998; 273(48): 32304-32311.

**The Gene for Recessive Polycystic Kidney Disease is Mapped.** The PKHD1 (polycystic kidney and hepatic disease 1) gene responsible for autosomal recessive polycystic kidney disease (ARPKD) has been mapped. ARPKD involves the kidneys and the biliary tract with an estimated incidence of 1 in 20,000 live births. The development of this high-resolution physical

map of the PKHD1 region is essential to cloning the PKHD1 gene and identifying the defect that leads to the disease.

Park JH et al., *A 1-Mb BAC/PAC-based physical map of the autosomal recessive polycystic kidney disease gene (PKHD-1) region on chromosome 6.* , Genomics 1999; 57(2):249-55.

**Identification of a Membrane Protein That Protects Against the Inflammation of Glomerulonephritis.** This study examined the role of P-selectin, a membrane protein expressed on the surface of activated endothelial cells (cells that line blood vessels) and platelets in glomerulonephritis, an immunologically-mediated kidney disease. The glomerulus is that portion of the functional unit of the kidney that filters the blood. Using mice that are deficient in P-selectin, the researchers found that the deficient mice exhibited more severe glomerular damage, and subsequent mortality when compared to normal mice. P-selectin on the endothelial cells was predominantly responsible for protection from the exacerbated disease, because mice with endothelial P-selectin, but not mice with platelet P-selectin, showed glomerular injury similar to that in normal. Levels of soluble circulating P-selectin were increased in normal mice and in mice with endothelial P-selectin, but not in mice with platelet P-selectin. Levels of soluble P-selectin, which has been shown to be anti-inflammatory *in vitro*, were inversely associated with the severity of disease. Thus, the protective effect in normal (wild-type) mice may be accounted for, in part by soluble P-selectin shed by non-renal endothelial cells. [secondary B treatment and prevention]

Rosenkranz AR, Mendrick DL, Cotran RS, Mayadas TN, *P-selectin deficiency exacerbates experimental glomerulonephritis: a protective role for endothelial P-selectin in inflammation.* J. Clin Invest 1999 Mar;103(5):649-59

**Diamond-Blackfan Anemia: The First Human Disease Caused by a Mutation in a Gene for a Ribosomal Protein.** Diamond-Blackfan Anemia (DBA) is an anemia with decreased or absent red blood cell precursor cells (erythroblasts) in the bone marrow, but otherwise normal cells. Until the present study, the basic genetic molecular defect in DBA has remained unclear. A gene responsible for DBA was previously mapped genetically by these investigators. They now report that the critical gene encodes the ribosomal protein S19. Ribosomes are sites of protein synthesis in the cytoplasm of the cell that provide the structural backbone for the synthesis in response to genetic instructions. By sequencing the ribosomal protein S19, the researchers identified mutations in 25% of unrelated DBA patients (10 of 40). Although there have been speculations that mutations in ribosomal proteins can cause human disease, this is the first direct demonstration. Thus, this is the first reported identification of a human disease caused by mutations in a ribosomal protein. [secondary B treatment and technologies]

Draptchinskaia N et al., *The ribosomal protein S19 gene is mutated in Diamond-Blackfan anemia.* Nature Genetics 21: 169-175, 1999.

**The Immunosuppressant Cyclosporine Causes Cancer Progression.** Malignancy is a common and dreaded complication following organ transplantation. The high incidence of neoplasm and its aggressive progression, which are associated with immunosuppressive therapy, are thought to be due to the resulting impairment of the organ recipient's immune-surveillance system. Researchers investigated the mechanism for the heightened malignancy that is independent of host immunity. They showed that cyclosporine (cyclosporin A), an immunosuppressant that has had a major impact on improving patient outcome following organ transplantation, induces changes in cells, including invasiveness of normal (non-transformed) cells. In cancer cells, cyclosporin A caused striking morphological and functional changes associated with increasing malignancy of the cells. These changes are prevented by treatment with (monoclonal) antibodies directed at the growth factor known as transforming growth factor-beta (TGF-beta). In animals, cyclosporine-enhanced tumor growth in (severe combined) immunodeficiency mice (SCID-beige mice); whereas anti-TGF-beta antibodies prevented the cyclosporine-induced increase in the number of metastases. Their findings suggest that immunosuppressants like cyclosporine can promote cancer progression by a direct cellular effect that is independent of its effect on the host's immune cells, and that this effect involves TGF-beta production. [secondary B prevention and treatment]

Hojo M et al., *Cyclosporine induces cancer progression by a cell-autonomous mechanism.*, Nature 1999; 397(6719):530-4

**A Cloned Zebrafish Gene Is A Model for Human Congenital Anemia.** Mutation studies in the zebrafish have proven to be a good approach to finding the genes which can cause anemia in humans; blood formation in the fish is very similar to the human with regard to which genes become activated (gene expression) and to gene function. A screen that was done detected five gene groups that cause anemias characterized by small red cells with low hemoglobin (microcytic hypochromic anemias). Investigators describe a mutant in one of these five, called *Asauternes*,<sup>@</sup> abbreviated *sau*. *Sau* causes microcytic, hypochromic anemia, suggesting that hemoglobin production is perturbed. During embryogenesis, *sau* mutants have delayed red blood cell maturation and abnormal hemoglobin gene expression. Using cloning techniques, the investigators have shown that *sau* codes for the red blood cell-specific form of the enzyme aminolevulinic acid synthase (ALAS2; also known as ALAS-E), the enzyme required for the first step in heme biosynthesis (for hemoglobin). Inasmuch as mutations in ALAS2 cause a congenital form of anemia in humans (congenital sideroblastic anemia), *sau* represents the first animal model for study of this disease.

Brownlie A et al., *Positional cloning of the zebrafish *sauternes* gene: a model for congenital sideroblastic anemia.* Nat Genet 1998; 20:244-250.

**A Red Blood Cell Membrane Protein is Important for Normal Membrane Integrity.** This paper identifies for the first time a regulatory role for a red blood cell membrane protein in the transport of ions. A protein 4.2<sup>@</sup> is a major component of the red blood cell (RBC) membrane structure. Defects in protein 4.2 in humans give rise to anemias due to RBC destruction

(hemolytic anemias) of varying severity. In this study, targeted gene mutations were used in mouse embryonic stem (ES) cells to determine protein 4.2 functions *in vivo*. Protein 4.2-deficient mice (due to targeted mutation in the stem cell) were found to have shrunken RBCs, which altered the potassium/sodium content of the cells. Thus, protein 4.2 is important in the maintenance of normal RBC surface area and for normal RBC potassium and sodium transport. This finding is likely to have important implications for our understanding of ion transport mechanisms in various cells and tissues. [secondary B treatment]

Peters LL et al., *Mild spherocytosis and altered red cell ion transport in protein 4.2-null mice*. J Clin. Invest. 103: 1527-1537, 1999.

**Role of Niemann-Pick C1 Protein In Disease.** Niemann-Pick type C (NPC) disease is an inherited lipid storage disorder that affects the abdominal organs and central nervous system. A characteristic feature of NPC cells is the accumulation of low density lipoprotein-derived cholesterol in the lysosome, minute bodies found within many cell types. A gene that is mutated in human NPC disease, named NPC1, was recently identified. NIH investigators, using a model cell line displaying the NPC cholesterol-trafficking defect, have conducted experiments to identify structural features of the human NPC1 protein that are critical for its action on cholesterol trafficking within a cell. Data indicates that the movement of NPC1 protein to the cholesterol-laden lysosomal compartment of a cell is essential for expression of its biological activity and that certain portions of the NPC1 protein are critical for mobilization of cholesterol from lysosomes. These studies provide important insight into the complexities of cholesterol transport within cells.

*Niemann-Pick C1 Protein: Obligatory Role for N-terminal Domains and Lysosomal Targeting In Cholesterol Mobilization.* Watari H et al. PNAS 1999; 96(3); 805-810.

**Is a Low Leptin Concentration the Expression of the "Thrifty Genotype?"** The high prevalence of obesity and type 2 diabetes in some populations is believed to be the expression of a "thrifty genotype," which conferred survival advantages during periods of harsh environmental conditions, but has become a liability in industrialized environments of abundance. Leptin concentrations are a measure of fat metabolism and its regulation. Leptin was discovered as the product of the obesity gene in animals, and plays a prominent role in humans as well, regulating energy metabolism by sending signals within the brain and between the brain and the body. Low plasma leptin concentrations and a low metabolic rate may be the phenotypic expression of this genotype, but they may also be affected by behavioral and environmental factors. To determine whether genetic or environmental influences, or both, affected leptin concentration, NIDDK researchers first hypothesized that plasma leptin concentrations and resting metabolic rate would be lower in Mexican Pima Indians not yet exposed to an affluent lifestyle than in non-Pima Mexicans living in the same environment. They studied 208 nondiabetic Pima Indians living a traditional lifestyle in a remote, mountainous area of northwest Mexico and 183 nondiabetic non-Pima living in the same environment. They found no significant difference in plasma leptin concentration between the groups, even after adjustment for percentage body fat, waist

circumference, age, and sex. This suggests that leptin levels are not due to the expression of the thrifty genotype. Researchers then examined 224 Mexican Pima Indians living a remote, traditional lifestyle in an area of northwest Mexico and 418 U.S. Pima Indians living a North American lifestyle on the Gila River Indian Reservation in Arizona. Here their hypothesis was that leptin concentrations would be lower in Mexican Pima Indians because of their lower percent body fat, but could be influenced further by their lifestyle, independent of body composition. They found that overall, independent of body fat, Mexican Pima Indians had the highest leptin concentrations. Results from this study suggest that independent of body composition, leptin concentration may be increased by environmental factors, such as high carbohydrate diet and a high level of physical activity.

Is a Low Leptin Concentration, a Low Resting Metabolic Rate, or Both the Expression of the "Thrifty Genotype"? Results from Mexican Pima Indians. Fox CS et al. Am J. Clin. Nutr 1999; 68; 1053-1057

*Plasma Leptin Concentrations in Pima Indians Living in Drastically Different Environments.* Fox CS et al. Diabetes Care 1999; 22(2); 413-417

**It Caught My Eye.** The decision to focus the eyes on one of two targets begins with the sighting of both targets (sensory input) and ends with the coordinated movement of the eyes to focus on one target (motor outcome). The milliseconds between sensory input and motor output are more than a simple knee jerk reflex response as once thought. Primates trained to respond to visual cues demonstrate that anticipation of a reward, based on past experience, or the relative size of the reward associated with the target greatly influences target selection. The decision-making process is correlated with the increased firing of neurons in the sensory-motor processing area of the central nervous system. This provides an important framework to model and understand the neurophysiological basis of visual-guided behaviors.

Platt ML and Glimcher PW: Neural correlates of decision variables in parietal cortex. Nature 400:233-238, 1999.

Nichols MJ and Newsome WT: Monkeys play the odds. Nature 400:217-218, 1999.

**Understanding Cataract Formation.** Access to genetic tools has offered fresh approaches to understanding cataracts, a problem that affects over 13 million Americans. Scientists recently discovered that a mutation in a gene encoding a protein called osteonectin or SPARC (secreted acidic protein rich in cysteines) that is required for cell-to-cell communication causes congenital cataracts. When a mutant form of this gene is introduced into mice, the mice are born with cataracts. Because this protein's structure and function have been characterized, it may now be possible to develop a better understanding of the pathogenesis of cataracts.

Kantrow M, Kays T, Horwitz J, Huang Q, Sun J, Piatigorsky J and Carper D: Differential display detects altered gene expression between cataractous and normal human lenses. Invest Ophthalmol Vis Sci 39(12):2344-2354, 1998.

Kantrow M, Horwitz J and Carper D: Up-regulation of Osteonectin/SPARC in age-related cataractous human lens epithelia. Molecular Vision 4:17-23, 1998.

**Specificity in Visual Signaling Pathways.** Signaling molecules can be organized into different pathways within the same cell. Assembling these signaling molecules into architecturally-defined complexes is emerging as an essential cellular mechanism to ensure the specificity and selectivity of signaling. Recently, an NEI-funded scientist found that a *Drosophila* protein, InaD, functions as a multivalent scaffold molecule. InaD brings together several components of the phototransduction cascade into a macromolecular complex. This assembly was found to be important both for permitting a high concentration of transduction molecules in small domains and for allowing effective and specific signaling in the process of vision.

Zucker CS and Ranganathan R: The path to specificity. Science 283:650-651, 1999.

Scott K and Zuker CS: Assembly of the *Drosophila* phototransduction cascade into a signalling complex shapes elementary responses. Nature 395:805-808, 1999.

**Organization of Neurons in the Cerebral Cortex.** One of the major issues in understanding the function of the cerebral cortex of the brain is how the activity of populations of neurons are coordinated to produce a coherent output. Studies of single neurons in the monkey brain show that active populations of neurons shift from an averaging mode to a winner-take-all mode as the visual stimulus conditions controlling eye movements change. These experiments show that as the neuronal activity changed, the type of eye movement generated also changed indicating that the type of movement generated can be accounted for by the shifts in overlapping activity of cortical neurons.

Recanzone, GH and Wurtz RH: Shift in smooth pursuit initiation in MT and MST neuronal activity under different stimulus conditions. J Neurophysiol (In press).

**Analysis of Visual Motion.** The human visual system distinguishes between the motion of objects in the world about us and the motion that results because we ourselves move about in that world. An area of the cerebral cortex of the monkey, whose motion perception is very similar to that of humans, has been identified, which is particularly appropriate for seeing the motion of objects because neurons in this area both differentiate the object from its surround and they separate objects moving at different distances from the observer. Because previous experiments had identified areas related to motion generated by observer motion, the present experiments show that both types of motion (object and observer) might be represented in different areas of cerebral cortex.

Eifuku SE and Wurtz RH: Response to motion in extrastriate area MST1: disparity sensitivity. J Neurophysiol (In press).

**Rapid Visual Guidance of Movement.** As we move through the environment, we experience highly stereotyped patterns of visual motion, called optic flow. Recent studies of eye movements indicate that our brains are able to process optic flow information very rapidly, permitting

automatic adjustments of gaze without the subject having to think about it. These reflex-like behaviors use sensory-motor linkages that are known to involve particular regions of the cerebral cortex and are helping us to understand how the cortex processes visual information.

Miles FA: Short-latency visual stabilization mechanisms that help to compensate for translational disturbances of gaze. Ann NY Acad Sci 871:260-271, 1999.

**Mathematical Modeling of Rapid Eye Movements.** When we read or look around, our eyes make rapid movements (called saccades) that point at objects of interest so that we may see them more clearly. If these saccades are not accurate, or if the eyes continue to drift after them, vision is significantly impaired. Many areas of the brain must cooperate to make accurate, drift-free saccades. Researchers have built mathematical models of the functions performed by these different areas to allow us to understand how the brain converts visual information into an eye movement model. Such an understanding is essential when dealing with abnormal saccades caused by disease, drugs, trauma, or aging.

Quaia C, Lefevre P, and Optican LM: Model of the control of saccades by superior colliculus and cerebellum. J Neurophysiol 82(2):999-1018, 1999.

**Attentional Activity in the Cerebral Cortex.** Although the entire visual environment is represented on the retina, by the time this information reaches the region of the brain that interprets all of this information, the parietal association cortex or lateral intraparietal area, only salient and important objects are represented. Scientists have found that neurons in this area of the monkey cortex respond to stimuli that serve as the targets for eye movements or direct eye movements away, showing that this activity is related to the salience of the stimuli but not to any motor plan the stimuli may evoke. This information suggests that the main function of the parietal areas of the brain may be to describe the world around us, while other areas of the brain may help in planning how and when to act in that world.

Gottlieb J and Goldberg ME: The activity of neurons in the lateral intraparietal area of the monkey during an antisaccade task. Nature (In press).

**Pregnancy and Autoimmune Retinal Disease.** Some autoimmune diseases recede temporarily during pregnancy, while others are exacerbated. Scientists have studied the effect of pregnancy on autoimmune eye disease in a model of experimental autoimmune uveitis (EAU) in mice. They showed that pregnancy protected mice from development of uveitis. More detailed studies of the mechanism showed that pregnancy not only prevented generation of the autoimmune lymphocytes, but also suppressed the function of previously generated autoimmune effectors. This occurred without induction of an overall immune deficit and appeared to be mediated, at least in part by pregnancy-related temporary upregulation of the well known regulatory mediator transforming growth factor beta. These data help to clarify the complex regulatory relationships between the immune and endocrine systems and help explain clinically-observed remissions of

autoimmune diseases like arthritis, uveitis, and multiple sclerosis during pregnancy. These findings may also suggest new approaches to treatment. [secondary B treatment]

Agarwal RK, Chan CC, Wiggert B, and Caspi R: Pregnancy ameliorates induction and expression of experimental autoimmune uveitis. J Immunol 162:2648-2654, 1999 (In press).

**Hereditary Factors Affecting Predisposition to Uveitis.** Scientists have used a rat model of experimental autoimmune uveitis (EAU) to identify the genetic factors predisposing to uveitis, a sight threatening inflammation in the eye. By studying the segregation of known genetic markers with ability to develop disease in the progeny of a resistant and susceptible rat strain, several genetic regions were mapped that control EAU susceptibility. These regions contain a number of immunologically relevant loci that may be connected to susceptibility to other autoimmune and inflammatory diseases in animal models and in man. The study of these genes may provide insights into mechanisms that control uveitis and other human autoimmune and inflammatory diseases, and may identify novel targets for therapy.

Sun SH, Silver PB, Du Y, Caspi RR, Wilder RL and Remmers EF: Genetic analysis of Experimental Autoimmune Uveoretinitis (EAU) in rats. Int Immunol 11(4): 529-534, 1999.

**New Insights into Hereditary Eye Tumors.** Von Hippel-Lindau (VHL) disease is a hereditary cancer syndrome in which affected individuals are at risk of developing vascular-like tumors in the eye, brain, kidneys, pancreas, and ear. In the eye these tumors can cause massive bleeding and loss of vision. Now, for the first time scientists have used microdissection and gene analysis to demonstrate the loss of the VHL gene in the tumor cells of affected eyes. The tumor cells produce large amounts of protein that cause abnormal vessel growth. Therefore, a vascular-like tumor is formed in the eye and other organs. These findings help explain the mechanism for abnormal vessel growth (vascular tumor, angioma) and the appearance of tumor in the eye.

Chan CC, Vortmeyer AO, Chew EY, Green WR, Matteson DM, Shen DF, Linehan WM, Lubensky IA, Zhuang Z: VHL gene deletion and enhanced VEGF gene expression detected in the stromal cells of retinal angioma. Arch Ophthalmol 117: 625-630, 1999.

Vortmeyer AO, Chan CC, Chew EY, Matteson DM, Shen DF, Wellmann A, Weil R, Zhuang Z: Morphologic and genetic analysis of retinal angioma associated with massive gliosis in a patient with von Hippel-Lindau disease. Graefes Arch Clin Exp Ophthalmol 237:513-517, 1999.

**Novel Roles of GDF-9 in Regulating Fertility.** Female mice that did not have the gene for growth differentiation factor-9 (GDF-9) failed to develop eggs that could be fertilized. This finding opens the possibility of regulating fertility by enhancing or interrupting the biological action of GDF-9, or applying GDF-9 to identify causes of infertility related to ovarian function.

Elvin JA, Yan C, Wang P, Nishimori K, and Matzuk MM: Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. Molecular Endocrinology 13: 1018-1034, 1999.

Elvin JA, Clark AT, Wang P, Wolfman NM, and Matzuk MM: Paracrine actions of growth differentiation factor-9 in the mammalian ovary. Molecular Endocrinology 13: 1035-1048, 1999.

**Understanding Early Miscarriage.** NIH grantees at Northeastern University using a mouse model have discovered a version of a gene that makes early embryos, called zygotes, more likely to survive until they can implant in the uterus. The version of the gene, dubbed *Ped fast*, also allows the cells of the zygote to divide more rapidly than do zygotes having a different version of the gene. The researchers hope that identifying the human form of the gene may lead to better treatments for recurrent early miscarriage.

Wu I, Feng H, and Warner CM: Identification of two major histocompatibility complex class Ib genes, *Q7* and *Q9*, as the *Ped* gene in the mouse. Biol Reprod 60: 1114-1119, 1999.

**Environment and IQ Level.** NIH-funded researchers examined the nature-nurture issue by studying how the level of the parent-s education might affect their child-s verbal IQ. The study, part of the National Longitudinal Survey of Adolescent Health, found that the level of parental education moderated both genetic and shared environmental influences on the child-s verbal IQ. The researchers also found that IQ level was highly responsive to environmental variation in families with poor environments.

Rowe DC, Jacobson KC, and Van den Oord EJCG: Genetic and environmental influences on vocabulary IQ: parental education level as moderator. Child Development; In press.

**The Role of Hormones in Maternal Behavior.** Scientists funded by the NIH have identified specific brain regions in rats that appear to control maternal behavior, as well as two hormones that stimulate these brain areas. The hormones, prolactin and oxytocin, appear to stimulate portions of the brain structure known as the hippocampus. Apparently, the hormones act independently of each other, presumably as failsafe mechanisms: if one hormone system fails to function, the other will be able to take its place. This research may lead to ways to identify animals, and, ultimately, humans, at risk for abnormalities or deficiencies in mothering behavior.

Bridges RS: The genetics of motherhood. Nature Genetics 20: 108-9, 1998.

Bridges RS, Mann PE, and Coppeta JS: Hypothalamic involvement in the regulation of maternal behaviour in the rat: inhibitory roles for the ventromedial hypothalamus and the dorsal/anterior hypothalamic areas. Journal of Neuroendocrinology 11: 259-266, 1999.

**Healing Damaged Spinal Cords.** NIH grantees have accomplished the first steps toward the eventual goal of healing damaged spinal cords. Previously studies revealed that molecules known as neurotrophins help damaged areas in the brain to repair themselves, and that neurotrophins also appear to repair damaged spinal cord tissue. Now, the investigators have determined that spinal cord cells known as glial cells produce neurotrophins. By learning more

about how these glial cells function, researchers may gain insights that will allow them to regenerate injured spinal tissue.

[secondary B treatment]

Dreyfus CF, Dai X, Lercher LD, Racey BR, Friedman WJ, and Black IB: Expression of neurotrophins in the adult spinal cord in vivo. J Neuroscience Res 56: 1-7, 1999.

**A Mouse Model of Host Defense Against Lung Inflammation.** NIH-supported investigators have recently implicated the immune molecule called tumor necrosis factor (TNF) in lung inflammation and injury, specifically in tuberculosis. The researchers have developed a mouse model to help understand the possible mechanisms for the host's response to the disease. These mice can be a useful tool for examining how the lung reacts to the disease-causing organisms.

Smith S, Skerrett SJ, Chi EY, Jonas M, Mohler K, and Wilson CB: The locus of tumor necrosis factor-alpha action in lung inflammation. Am J Respir Cell Mol Biol 19: 881-891, 1998.

**New Ways to Reduce Jet Lag on the Horizon.** NIH researchers have taken the first preliminary steps toward the eventual design of drugs to prevent jet lag and to maintain wakefulness in those who need to remain awake at night. Specifically, the researchers have developed molecules that inhibit the enzyme known as arylalkylamine N-acetyltransferase, which breaks down melatonin, the molecule that regulates sleeping and waking. Earlier, the group had discovered the gene for the enzyme, as well as how the enzyme is broken down inside barrel-shaped structures known as proteasomes. [secondary B treatment]

Fleming JV, Barrett P, Coon SL, Klein DC, and Morgan PJ: Ovine arylalkylamine N-acetyltransferase in the pineal and pituitary glands: differences in function and regulation. Endocrinology 140: 972-978, 1999.

Hickman AB, Klein DC, and Dyda F: Melatonin biosynthesis: the structure of serotonin N-acetyltransferase at 2.5 Å resolution suggests a catalytic mechanism. Molecular Cell 3: 23-32, 1999.

Hickman AB, Nambodiri MAA, Klein DC, and Dyda F: The structural basis of ordered substrate binding by serotonin N-acetyltransferase: enzyme complex at 1.8Å resolution with a bisubstrate analog. Cell 97: 361-369, 1999.

**Genes That Control Early Brain Development.** Using gene "knockout" technology in mice, NIH scientists have discovered several genes controlling early brain development, in various parts of the pituitary, forebrain, and hippocampus. In recent years, NIH researchers have identified several genes controlling development of the pituitary gland, which regulates many of the body's hormonal systems, and of the forebrain, responsible for thought and coordination. They also have discovered the gene that controls development of the hippocampus, the brain structure playing a large role in learning and memory. The results of these basic studies may one

day lead to greater understanding of how the brain contributes to normal and abnormal functioning.

Zhao Y, Sheng HZ, Amini R, Grinberg A, Lee E, Huang SP, Taira M, Westphal H: Control of hippocampal morphogenesis and neuronal differentiation by the LIM homeobox gene *Lhx5*. Science 284:1155-1158, 1999.

**Powerful Anti-AIDS Agent Found in Tears and Urine of Pregnant Women.** A research team from NIH and New York University have found that tears, saliva and pregnant women's urine contain proteins that were able to kill the AIDS virus quickly in test-tube experiments. The proteins could lead to an important drug against HIV because they are a natural compound that the body may tolerate with fewer side effects. While it is not known how the proteins kill the HIV, researchers speculated that they may break down the outer membrane of the virus. [secondary B treatment]

Lee-Huang S, Huang Paul L, Sun Y, Huang Philip L, Kung H-F, Blithe DL, and Chen H-C: Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin. Proc Natl Acad. Sc 96: 2678-81, 1999.

**Maternal Caffeine Use and the Outcome of Pregnancy.** The issue of whether caffeine consumption during pregnancy increases the risk of spontaneous abortion is controversial. Some studies have suggested a greater risk of miscarriage from caffeine consumption during pregnancy. However, researchers working at the NIH and the University of Utah found that a moderate amount of caffeine, the amount of caffeine consumed by most pregnant women, does not cause miscarriage. Nevertheless, women who consume extraordinarily large amounts of caffeine may increase their risk.

Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, and Wilkins DG: Maternal serum caffeine metabolites and the risk of spontaneous abortion. NEJM; In press.

**A Better Way for Nurses to Assess Infant Pain.** Scientists have found an improved method of identifying pain in infants. Careful observation of how the infant responds to comfort measures can help nurses and others identify when and how much pain infants experience, both before and following intervention for pain. The new method of pain assessment makes it less likely that infants will be undertreated for pain, or overtreated for pain with analgesic medication when their behavior is incorrectly assessed as being pain-related.

Fuller BF, Neu, M, Smith, M, Vojir, CP: Testing a model of the nursing assessment of infant pain. Clinical Nursing Research 8:1, 69-83, 1999.

**Clue to Excess Prevalence of High Blood Pressure in Blacks.** New research findings may help to explain why blacks experience a higher prevalence of hypertension and its complications than whites. In a study in which both blacks and whites with normal blood pressure were subjected to mental stress, blood vessels that were narrowed as a result of the mental stress

opened at a slower rate in the black subjects than in their white counterparts. In related studies, the same investigators determined that the difference may be related to a decreased effect in blacks of nitric oxide, a substance that relaxes the smooth muscle in blood vessels. This new knowledge may provide a basis for developing prevention and treatment strategies to reduce the adverse health impact of hypertension in blacks. [secondary B treatment]

Cardillo C, Kilcoyne CM, Cannon RO, Panza JA: Racial differences in nitric oxide-mediated vasodilator response to mental stress in the forearm circulation. Hypertension 31:1235-1239, 1998.

Cardillo C, Kilcoyne CM, Cannon RO, Panza JA: Attenuation of cyclic nucleotide-mediated smooth muscle relaxation in blacks as a cause of racial differences in vasodilator function. Circulation 99:90-95, 1999.

**A New Lesson From Sleeping Dogs.** All dogs sleep a lot, but dogs with narcolepsy, like people with narcolepsy, are subject to daytime sleepiness, sudden muscular weakness, and rapid onset of deep sleep. Because canine narcolepsy shows close similarity to human narcolepsy, the recent finding of the genetic mutation that causes canine narcolepsy offers great promise for improved understanding of the human form of the condition and may lead to potential approaches to treatment.

Chemelli RM, Willie JT, Sinton CM et al: Narcolepsy in *orexin* knockout mice: molecular genetics of sleep regulation. Cell 98:437-451, 1999.

Lin L, Faraco J, Li R et al: The sleep disorder canine narcolepsy is caused by a mutation in the *hypocretin (orexin) receptor 2* gene. Cell 98:365-376, 1999.

**Inflammatory Findings on Diabetes.** A number of clinical markers of a general inflammatory response, including elevated white blood cell counts and elevated fibrinogen levels, have been found to be present in persons who develop diabetes an average of 7 years before their disease is diagnosed. If confirmed, these results could offer an additional approach beyond weight control for diabetes prevention. Sources of inflammation, whether infectious or due to environmental factors such as cigarette smoking, could be identified and efforts undertaken to control them or mitigate their effects. [secondary B prevention]

Schmidt MI, Duncan BB, Sharret AR et al: Markers of inflammation and prediction of diabetes mellitus in adults (atherosclerosis risk in communities study): a cohort study. The Lancet 353:1649-52, 1999.

**Homocysteine: Another Kind of Heart Risk.** Homocysteine, a by-product of protein metabolism that is produced in the body when the diet lacks sufficient folic acid (a B vitamin), has only recently been widely accepted as a risk factor for atherosclerosis. Now it appears that homocysteine is implicated in the class of human birth defects that includes certain anomalies of heart development, as well as spina bifida, anencephaly, and orofacial clefts. Investigators have established that homocysteine can disrupt the function of a protein found on cell membranes that plays a key role in normal developmental pathways during early embryonic stages.

Andaloro VJ, Monaghan DT, Rosenquist TH: Dextromethorphan and other N-methyl-D-aspartate receptor antagonists are teratogenic in the avian embryo model. *Pediatric Research* 43:1-7, 1998.

Rosenquist TH, Andaloro VJ, Schneider AM, Monaghan DT: N-methyl-D-aspartate receptor agonists modulate homocysteine-induced developmental disorders. *FASEB Journal*, in press, 1999.

**Too Much Ado About Mitral-Valve Prolapse.** A recent report from the Framingham Heart Study should relieve many of the concerns that young women may have about a condition known as mitral-valve prolapse (MVP) and its association with such serious clinical conditions as stroke, heart attack, and heart failure. Whereas previous reports characterized MVP as a common condition, with prevalence among young women ranging as high as 10-15 percent, its overall prevalence in a selection of patients from the Framingham study was only 2.4 percent. Moreover, those with MVP were no more likely to have experienced stroke, heart attack, heart failure, or atrial fibrillation than persons of comparable age and sex without the condition. [secondary B prevention]

Freed LA, Levy D, Levine RA et al: Prevalence and clinical outcome of mitral-valve prolapse. *NEJM* 341:1-7, 1999.

**New Insights into Human Cell Aging.** Research has demonstrated that the Ras gene, a gene implicated in many cancers, may also play a role in the aging process of human cells. When a form of the Ras gene that is continually active was introduced into cultures of human cells, investigators noted an acceleration of the aging process and reduced cell life span, apparently due to increased levels of a reactive form of oxygen known as free radicals. Investigators have now identified a cellular component known as the mitochondria as the source of the reactive oxygen form, and identified a class of compounds that appear to reduce its production by the mitochondria without being toxic to the cells themselves. The compounds may thus ultimately provide insight into ways to slow the aging process. [secondary B prevention]

Lee AC, Fenster BE, Ito H et al: Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *Journal of Biological Chemistry* 274:7936-7940, 1999.

**Declining Seroprevalence in New York City HIV Epidemic.** As the city that has experienced the largest AIDS epidemic among injection drug users (IDUs) anywhere in the world, researchers report that New York City is beginning a new phase of the HIV epidemic among IDUs. Data collected from 1991-1996 show that HIV seroprevalence declined substantially among the subjects in five studies conducted throughout the city. Potential reasons for these reductions may be the loss of HIV-seropositive individuals through disability and death and lower rates of risk behavior leading to low HIV incidence.

Des Jarlais, DC, Perlis T, Friedman SR, Deren S., et al. "Declining Seroprevalence in a Very Large HIV Epidemic: Injecting Drug Users in New York City, 1991 to 1996." *Am J Public Health* 88(12): 1801-1806, 1998.

**Changing Drug Use Patterns.** Researchers looking at risk behavior and HIV infection among new drug injectors in the New York City population found that new injectors appear to have adopted the reduced risk practices of long-term injectors in the city. In general, new initiates to drug injection (injecting from 0 up to 6 years) were found more likely to be female and white. The new initiates were also found to have begun injecting at an older median age of 27 years and found to have substantial prevalence of HIV infection (11% among persons injecting up to 3 years, 18% among persons injecting 3 to 6 years). From a public health perspective, this change in drug use patterns demonstrates the need for programs designed to reduce initiation into drug injection, rather than simply relying on the fear of AIDS as a deterrent.

Des Jarlais, DC, Friedman SR, Perlis T, Chapman T., et al. "Risk Behavior and HIV Infection Among New Drug Injectors in the Era of AIDS in New York City." J AIDS and Human Retrovirol 20(1): 67-72, 1999.

**Who's Using Methamphetamine? New Insight.** With methamphetamine use reaching epidemic proportions in many parts of the country, researchers are trying to better understand the populations that use this stimulant. A study of methamphetamine and heroin injectors in San Antonio, Texas found that methamphetamine users were younger (30) than those who took heroin, were more likely to be white and had less severe drug dependence. Methamphetamine users also were found to have lower needle risk behaviors based on findings that they tended to buy 10 or more syringes at a time and to reuse syringes less frequently than those who were addicted to heroin.

Zule WA, Desmond DP, An ethnographic comparison of risk behaviors among heroin and methamphetamine injectors. Am J Drug Alcohol Abuse 25(1): 1-23, 1999.

**The Environment of the Mouse Matters.** A great deal of effort is being devoted to identifying genes that influence behaviors such as addiction, cognition, personality, and various psychiatric disorders to name a few. These efforts just became more complicated. Researchers from three laboratories across North America applied the same battery of behavioral tests to the same genetically identical strains of mice, under almost the exact same circumstances---and got strikingly different results, demonstrating that small, undetectable variations in environment can have a significant effect on behavior. Based on these results, the role that a particular gene has on a behavior can be masked by environmental influences and therefore genetic effects on behavior should be confirmed in multiple labs, with multiple tests before concluding any positive linkage.

Crabbe JC, Wahlsten D, and Dudek BC: Genetics of mouse behavior: Interactions with laboratory environment. Science 284: 1670-72, 1999.

**Unraveling the Mysteries of Relapse.** Scientists have recently developed an animal model of drug craving that they are using to explore the brain circuitry that contributes to relapse. Animals trained to self-administer cocaine who are subsequently withdrawn from the drug for extended

periods can be triggered into relapse by either exposure to environmental cues associated with the drug-taking behavior or by administration of a low dose of the drug. The researchers found that different circuits in the brain may be involved in relapse triggered by exposure to the drug versus relapse triggered by environmental cues. These results suggest that treatment of relapse may differ depending on the triggering cues.

Tran-Nguyen L, Fuchs RA, Coffrey GP, Baker DA, O'Dell LE, Neisewander JL. Time-dependent Changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19: 48-59, 1998.

**Further Evidence of the Association between Human Papillomavirus Infection and Cervical Cancer.** Human papillomavirus (HPV) infection has been strongly associated with cervical cancer and its precursors, squamous intraepithelial lesions (SIL). In order to clarify the role of HPV in the etiology of SIL, researchers followed 17, 654 women whose Pap tests were normal, with no SIL evident; they found that women who tested positive for HPV DNA at the beginning of the study were 3.8 times more likely to have low-grade SIL subsequently diagnosed for the first time during follow-up and 12.7 times more likely to develop high-grade SIL. These findings are consistent with the hypothesis that HPV infection is the primary cause of cervical neoplasia and indicate the need for clinical studies on applications of HPV DNA testing and primary prevention of cervical cancer by vaccination.

Law KL, Glass AG, Manos MM, et al.: Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. Journal of the National Cancer Institute 91: 954-960, 1999.

**Breast Cancer Genetics.** Women who have inherited mutations in the BRCA1 gene have a greatly increased risk of developing breast cancer compared to the general population; however, other genes, particularly those involved in hormonal signaling, may modify BRCA1-associated age-specific breast cancer risk. Researchers studied the effect of a variation B repeats of the triplet nucleotide CAG B in the androgen-receptor gene that is associated with a decreased ability to activate androgen-responsive genes. They found that women with BRCA-1 mutations whose androgen receptor CAG triplet repeated 28 or more times were diagnosed with breast cancer significantly earlier than women whose CAG triplet repeated fewer than 28 times. These results support the hypothesis that age at breast cancer diagnosis is earlier among BRCA1 mutation carriers who carry very long androgen receptor CAG repeats, and suggest that pathways involving androgen signaling may affect the risk of BRCA1-associated breast cancer.

Rebeck TR, Kantoff PW, Krithivas K, et al.: Modification of BRCA1-associated breast cancer risk by the polymorphic androgen-receptor CAG repeat. American Journal of Human Genetics 64: 1371-1377, 1999.

**Mechanisms of Angiogenesis.** The formation of new blood vessels, a process referred to as angiogenesis, is a key event during tumor development and metastasis. Investigators elucidated the role of the compound SDF1-alpha in angiogenesis, and how it promotes tumor growth and

spread. Mechanisms of controlling levels of SDF-1 may be useful in cancer prevention and treatment.

Salcedo R, Wasserman K, Young HA, et al.: Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells. American Journal of Pathology 154: 1125-1135, 1999.

**Functions of BRCA1.** Researchers have gained critical insights into two major cancer-associated functions of BRCA1: a deficiency in cell cycle check points, and amplification of centrosomes the cellular structures that are involved in the organization of microtubules for the proper and equal distribution of the genetic material during cell division. Centrosomes are the cellular structures that are involved in the organization of microtubules for the proper and equal distribution of the genetic material during cell division; abnormalities in centrosomes may therefore explain one of the most commonly observed genetic abnormalities in cancer cells, that is, the presence of extra copies of chromosomes carrying oncogenes or the loss of chromosomes that harbor tumor suppressor genes.

Xu X, Weaver Z, Linke SP, et al.: Centrosome amplification and a defective G<sub>2</sub>-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Molecular Cell 3: 389-395, 1999.

**Identification of a Protein that Helps Maintain Genomic Stability.** Cells whose chromosomes behave normally are less likely to become cancerous. Investigators have determined the function of the protein Gadd45, which when properly regulated promotes chromosomal stability, but when lost leads to genomic instability C i.e., errors in genetic material that can lead to the formation of cancer.

Wang XW, Zhan Q, Coursen JD, et al.: GADD45 induction of a G<sub>2</sub>-M cell cycle checkpoint. Proceedings of the National Academy of Sciences 96: 3706-3711, 1999.

Zhan Q, Antinore MJ, Wang XW, et al.: Association with Cdc2 and inhibition of Cdc2/Cyclin B1 kinase activity by the p53-regulated protein GADD45. Oncogene 18: 2892-2900, 1999.

**Prostate Cancer Genetics.** NIH-supported researchers have identified an alteration -- a region of deletion -- on chromosome 8 (8p21) that existed in approximately 80 percent of prostate tumors studied. This region also overlaps a region recently identified as a locus for familial breast cancer. Deletions were also found in 63% of precancerous prostate lesions, suggesting that abnormalities on 8p21 may be associated with early stages of prostate cancer development. A physical map of this region is currently being constructed, and researchers have begun analyzing candidate genes that reside in this area.

Emmert-Buck M., Strausberg R, Schuler, et al.: Analysis of human prostate cancer gene expression by cDNA library sequencing. Nature Medicine, in press.

Cheng L, Song S-Y, Pretlow TG, et al.: Evidence of independent origin of multiple tumors from patients with prostate cancer. Journal of the National Cancer Institute 90: 233-237, 1998.

Afonso A, Emmert-Buck MR, Duray PH, et al.: Loss of heterozygosity on chromosome 13 is associated with advanced stage prostate cancer. Journal of Urology, in press.

Strup SE, Pozzatti RO, Florence CD, et al.: Chromosome 16 allelic loss analysis of a large set of microdissected prostate carcinomas. Journal of Urology 162: 590-594, 1999.

Chuaqui RF, Englert CR, Strup SE, et al.: Identification of a novel transcript up-regulated in a clinically aggressive prostate carcinoma. Urology 50: 302-307, 1997.

Cole KA, Chuaqui, Katz K, et al.: cDNA sequencing and analysis of POV1 (PB39): A novel gene up-regulated in prostate cancer. Genomics 51: 282-287, 1998.

**Function of the Met Gene in Hereditary Papillary Renal Carcinoma.** Researchers have made several important discoveries about the role of the proto-oncogene, Met, in Hereditary Papillary Renal Carcinoma (HPRC); HPRC is a hereditary cancer syndrome in which affected individuals are at risk for the development of a certain type of kidney cancer. They have found that the inherited Met mutation is an activating mutation, i.e. when a Met gene with the mutation is placed into cells grown in culture these cells now form tumors when injected into mice, whereas the parent cells did not. They have also found that there are three copies (trisomy) of the Met gene in kidney cancers that develop in affected individuals; this study demonstrated for the first time the non-random duplication of a human oncogene (Met) in a hereditary human cancer (HPRC).

Schmidt L, Junker K, Weirich G, et al.: Two North American families with Hereditary Papillary Renal Carcinoma and identical novel mutations in the MET proto-oncogene. Cancer Research 58: 1719-1722, 1998.

Zhuang Z, Park W-S, Pack S, et al.: Trisomy 7-harboring non-random duplication of the mutant MET allele in hereditary papillary renal carcinomas. Nature Genetics 20: 66-69, 1998.

**Hormone Levels, Dietary Soy, and Breast Cancer.** Results of three studies are reported here: In the first, investigators found statistically significant associations with risk of breast cancer and circulating levels of the hormones estradiol, estrone, estrone sulfate, and dehydroepiandrosterone sulfate; these associations were substantially stronger among women with no previous hormone replacement therapy, and the results suggest that high estrogen levels may increase breast cancer risk in postmenopausal women. In the second study, high levels of prolactin, a hormone essential for breast development and lactation, were found to increase postmenopausal breast cancer risk. Finally, levels of isoflavonoids, found in soy foods and hypothesized to reduce breast cancer risk, were substantially lower in the urine of women diagnosed with breast cancer than in other women; the results support the hypothesis that a high intake of soy foods may reduce breast cancer risk.

Zheng W, Dai Q, Custer LJ, et al.: Urinary excretion of isoflavonoids and the risk of breast cancer. Cancer Epidemiology, Biomarkers, & Prevention 8: 35-49, 1999.

Hankinson SE, Willett WC, Manson JE, et al.: Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. Journal of the National Cancer Institute 90: 1292-1299, 1998.

Hankinson SE, Willett WC, Michaud DS, et al.: Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. Journal of the National Cancer Institute 91: 629-634, 1999.

**What's the Natural Purpose of a Marijuana Receptor?** CB1 (cannabinoid receptor 1) appears to be the molecule in the brain that mediates the effects of marijuana, and scientists are curious as to the purpose of CB1's role in the normal brain *absent* marijuana. To address the question, NIH investigators genetically engineered a strain of mice that lacked the cannabinoid CB1 receptor. The mutant animals proved to be less active than normal mice and less motivated to avoid unpleasant stimuli; interestingly, knocking out CB1 blocked only some of the behavioral effects of marijuana, indicating that marijuana must interact with an undetermined number of molecular targets and pathways in the brain.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI: Increased mortality, hypoactivity and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proceedings of the National Academy of Science 96: 5780, 1999.

Steiner H, Bonner TI, Zimmer AM, Kitai ST, and Zimmer A.: Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. Proceedings of the National Academy of Science 96: 5786-5790, 1999.

**The Way We Were: Making Vs. Storing Long-term Memories.** Imagine a person who has suffered infectious illness of such severity that it virtually obliterated regions of the brain B the hippocampus and adjoining structures of the medial temporal lobe -- known to be essential to memory and who, in fact, is unable to recognize a visitor who had been to his home more than 40 times. Presented with the opportunity to interview just such a subject, NIH-funded investigators were intrigued to find that although the man was ignorant of his current neighborhood, to which he had moved after his illness, he possessed vivid, accurate recollections of the town in which he had grown up some five decades earlier. Results of this clinical study indicate that different brain structures are responsible for generating and for retrieving long-term memories.

Teng E, Squire LR: Memory for places learned long ago is intact after hippocampal damage. Nature 400: 675-677, 1999.

**Genetic Locus for Specific Language and Reading Deficits.** Reading disability, or dyslexia, is a complex cognitive disorder manifested by difficulties in learning to read in otherwise normal individuals. Recently, scientists have pinpointed the chromosomal location for a gene that influences reading disability. The putative genetic locus is quite close to the human leukocyte antigen region, which implies that a coding or regulatory gene related to the immune system influences reading deficits. [secondary B treatment]

Gayán J et al.: Quantitative-trait locus for specific language and reading deficits on chromosome 6p. Am J Hum Genet 64:157-164 1999.

**Competition Among Brain Chemicals Suggests New Path to Medication Development.**

Brains suffering from several neurodegenerative diseases including Alzheimer's disease, AIDS-dementia, and multiple sclerosis as well as ischemic insult may gain some protective benefit from a particular protein of the cytokine family, called insulin-like growth factor, or IGF-1. NIH scientists recently discovered, however, that the same molecular pathway responsible for IGF-1 action also involves a competing cytokine called tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) which has been shown to cause neuron death. The discovery suggests that the IGF-1 receptor and its molecular pathway may be a promising target for the development of new therapeutics which may prevent the neurodegeneration caused by TNF" in response to brain inflammation or injury. [secondary B treatment]

Venters HD, Tang Q, Liu Q, VanHoy RW, Dantzer R, Kelley KW: A new mechanism of neurodegeneration: A proinflammatory cytokine inhibits receptor signaling by a survival peptide. Proceedings of the National Academy of Sciences 96: 9879-9884, 1999.

**To Sleep, Perchance, To Have a Memory Work-out.** Research with birds has strongly endorsed a long-held hypothesis that one function of sleep is to strengthen some memories. NIH-supported investigators devised a way to study birds while they slept, and found that during sleep the brain circuits responsible for their daytime song get re-wired and strengthened. Even though the sleeping birds don't hear their song while they slumber, the song circuits repeat the same patterns experienced during wakefulness B a kind of unconscious practice to fine tune the neurons for the job ahead the next day. [secondary B prevention]

Dave AS, Yu AC, Margoliash D: Behavioral state modulation of auditory activity in a vocal motor system. Science 282: 2250-2254, 1998.

**Generational Transmission of Psychopathology.** In a multi-generational study, NIH-funded investigators found that, in families in which both grandparents and parents had experienced a clinical depression, a disconcerting 49% of the grandchildren showed signs of psychopathology, with high risk for anxiety. Typically, anxiety in young children is viewed to be a part of normal development, and something that the child will outgrow. In these high-risk families, however, anxiety symptoms are being transmitted across the generations, and, for family members, the onset of an anxiety disorder before puberty is linked to a high risk for the subsequent development of serious, recurring major depression. [secondary B prevention]

Warner V, Weissman MM, Mufson L, Wickramaratne PJ: Grandparents, parents, and grandchildren at high risk for depression: A three-generation study. Journal of the American Academy of Child and Adolescent Psychiatry, 38, 289-296, 1999.

**Delaying Menopause-Related Health Problems: Estrogen Protection With or Without Fertility.** Scientists have developed a transgenic mouse genetically altered to lack a gene called Abax.® This gene blocks a pathway for cell death so that eggs are lost from the mouse ovary much more slowly than in normal mice. The result is that the transgenic mice maintain function

of their ovaries much longer than normal, thus delaying the mouse equivalent of menopause. While estrogen-sensitive tissue in normal mice shrinks in advanced age due to the apparent loss of estrogen, these tissues remain youthful in the genetically altered mice. This study is the first to find that loss of ovarian function can be reduced and raises the possibility of delaying or preventing menopause-related changes in humans, for example by blocking the adverse effects of estrogen deficiency, including increased risk of heart disease and development of osteoporosis. [secondary B prevention]

Perez GI, Robles R, Knudson CM, Flaws J, Korsmeyer SJ, and Tilly J: Prolongation of ovarian lifespan into advanced chronologic age in Bax-deficiency. Nature Genetics 21: 200-203, 1999.

**Identified Protein May Lead to Gene Therapy for Huntington's Disease.** Several degenerative diseases of the neurologic system, including Huntington's Disease, are caused by expanded repetition of specific gene sequences. Transgenic mice expressing this same expanded gene repetition develop a syndrome with increasing age that resembles Huntington's disease. Researchers recently found that blocking an enzyme, caspase-1, involved in cell death pathways delayed the onset of symptoms and death in a mouse model of Huntington's disease by an average of 20 days. When a drug that inhibits the enzyme was continuously administered for 4 weeks, the mice suffered less disability and lived 25% longer than untreated mice. Since cell death pathways are shared by many neurodegenerative disease, inhibiting caspase-1 could improve the prospects for many such diseases, including Huntington's disease, and amyotrophic lateral sclerosis (Lou Gehrig's disease). [secondary B treatment]

Ona VO, Li M, Vonsattel MPG, Andrews LJ, Khan SQ, Chung WM, Frey AS, Menon AS, Li X, Steig E, Yuan J, Penney JB, Young AB, Cha JHJ, and Friedlander RM. Inhibition of caspase-1 shows disease progression in a mouse model of Huntington's disease. Nature 399: 263-267, 1999.

**Neighborhood and Socioeconomic Characteristics Make It Difficult to Initiate and Maintain Recommended Physical Activity Levels.** Recent analyses from the Alameda County Study show that neighborhood characteristics affect physical activity levels. Living in a poor neighborhood is associated with a decline in physical activity, even adjusting for age, individual income, education, smoking status, body mass index, and alcohol consumption. Other survey analyses reveal that poor weather and fear of crime were major barriers to exercise in low income urban older adults, as was the lack of information from physicians and family/friends regarding the safety and benefits of exercise. These studies demonstrate the importance of designing physical activity/exercise programs that can counter the negative effects of disadvantaged social conditions.

Yen IH, and Kaplan GA: Poverty area residence and changes in physical activity levels: evidence from the Alameda County study. American Journal of Public Health 88: 1709-12, 1998.

Clark DO, and Nothwehr F: Exercise self-efficacy and its correlates among socioeconomically disadvantaged older adults. Health Education and Behavior 26: 535-46, 1999.

**Centenarians Live Most of Their Lives in Good Health.** Scientists have found preliminary evidence that many centenarians remain functionally independent for the vast majority of their lives and then experience a relative rapid decline near the end of their lives. Relative to others in the older population, they also appear to either experience a marked delay in the onset or, in some cases, escape diseases such as cancer and Alzheimer's disease. Scientists also find a strong familial component to extreme longevity. Siblings of centenarians tend to live longer compared to siblings of individuals who died in their mid-70s. This may be due in part to shared genetic traits among family members. Understanding the genetic and environmental factors responsible for centenarians' prolonged good health could provide insights for improving the health of all older people.

Hitt R, Young-Xu Y, Silver M, and Perls T: Centenarians: The older you get, the healthier you've been. Lancet (forthcoming).

Perls T, Bubrick E, Wager CG, Vijg J, and Kruglyak L: Siblings of centenarians live longer. Lancet 351: 1560, 1998.

**Chronic Inflammation in the Elderly May Lead to Disability and Early Death.**

Inflammation is a normal biologic response to acute insults to the body. However, inflammation can become chronic and adversely affect aging and disease processes. Researchers have identified an immune system protein, interleukin-6, that plays a role in inflammation and is, therefore, a candidate risk factor for certain types of disabilities in older adults, including depression, heart failure, and arthritis. Participants in a study of men and women aged 70 and greater showed a significant correlation between interleukin-6 levels and a depression score. In another study in men and women 71 years or older, participants with the highest levels of interleukin-6 were almost twice as likely to develop mobility-disability and were about twice as likely to die within 5 years of the beginning of the study. Interleukin-6 stimulates the synthesis of C-reactive protein, an indicator of systemic inflammation. When levels of both interleukin-6 and C-reactive protein were elevated simultaneously, there was a 3-fold increased risk of mortality. These relationships suggest that a chronic inflammatory process may be contributing to age-related conditions. There is the possibility that high risk individuals could be identified who may respond to anti-inflammatory intervention. [secondary B prevention]

Dentino AN, Pieper CF, Rao KMK, Currie MS, Harris T, Blazer DG, Cohen HJ: Association of interleukin-6 and other biologic variables with depression in older people living in the community. J Am Geriatr Soc 47: 6-11, 1999.

Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti M, Cohen HJ, Penninx B, Pahor M, Wallace R, Havlik RJ: Serum IL-6 level and the development of disability in older persons. J Am Geriatr Soc 47: 639-646, 1999.

Harris TB, Ferrucci L, Tracy RP, Corti C, Wacholder S, Ettinger WH, Heimovitz H, Cohen HJ, Wallace R: Associations between elevated interleukin-6 and C-reactive protein levels and mortality in the elderly. The American Journal of Medicine 106: 506-512, 1999.

**Researchers Identify Genetic Mechanisms Involved in the Age-Related Increase of a Blood Clotting Factor.** Researchers have found a genetic mechanism through which production of a blood serum protein, factor IX, increases with age. This is important because the tendency to form blood clots increases with age and contributes to heart disease and stroke. Finding the genetic regulatory mechanism provides opportunities to design drugs or other interventions to prevent the increased likelihood of clotting and its associated diseases in older persons.

Kurachi S, Deyashiki Y, Takeshita J, and Kurachi K: Genetic mechanisms of age regulation of human blood coagulation factor IX. Science 285: 739-43, 1999

**Scientists Gain New Insights into Pathways Controlling Immune Function.** Older individuals, particularly those with diseases such as cancer, have a lower immune response than their younger counterparts. Scientists are seeking to understand all of the basic biochemical processes involved in the immune response in order to design appropriate therapies to help the body fight disease. One such biochemical pathway involves the role of protein tyrosine kinases. Intramural scientists have discovered that in human T lymphocytes, a type of cell involved in basic immune responses, the protein tyrosine kinase Itk is subject to regulation by another protein tyrosine kinase, ZAP-70. ZAP-70 indirectly activates Itk by regulating its interaction with another protein LAT (Linker for Activation of T-cells). These three proteins form a signaling chain, and it is likely that ZAP-70's role in regulating calcium mobilization in T cells comes from its regulation of Itk activity. These findings provide new insights into the tightly regulated signaling processes controlling T cell activation and may lead to new therapies to improve the immune response.

Shan X, and Wange RL: Itk/Emt/Tsk activation in response to CD3 cross-linking in Jurkat T cells requires ZAP-70 and Lat and is independent of membrane recruitment. Journal of Biological Chemistry, in press, 1999.

**Common Mechanism Is Identified for Gene-Specific and X Chromosome Inactivation.** Many diseases are associated with the turning on or off of specific genes. An understanding of the biologic processes involved can lead to development of therapies to control when genes get turned on or off and thus prevent the development of disease. Genes on the X chromosome can be turned off by two different mechanisms, either by gene-specific mechanisms on an active X or by a chromosome-wide inactivation mechanism on the second, inactivated X chromosome in every adult female cell. Intramural investigators have demonstrated for the first time that CpG dinucleotide clusters at the start of individual genes on the X chromosome are methylated to turn off the genes in both the gene-specific and chromosome-wide mechanisms. Thus, a common mechanism is shared between the two different inactivation processes, and occurs at the same locations in DNA. This knowledge could eventually lead to the development of therapies to control when genes get turned on or off.

Huber R, Hansen S, Strazzulo M, Pengue G., Mazzarella R, Gartler S, D-Urso M, Schlessinger S, Pilia G, and D-Esposito M: DNA Methylation in Transcriptional Repression of Two Differentially Expressed X-linked Genes, GPC3 and SYBL1. Proc. Natl. Acad. Sci. USA 96: 616-621, 1999.

**One Form of the ApoE Gene Protects Brain Cells from Injury.** Although the mechanism through which it works is unknown, the only accepted risk factor for sporadic late-onset Alzheimer's disease is the gene for apolipoprotein E4 (ApoE4). This study presents compelling evidence to suggest that the presence or absence of a particular ApoE structural variant or isoform affects the way neurons respond to injury. In these studies, which involved mice made transgenic for one or the other human ApoE isoform, it was determined that the ApoE3 isoform protected the brain against excitotoxic injury but that ApoE4 did not. The significance of this finding is that it may help to explain how ApoE4 is a risk factor for the development of AD, and, if confirmed, might suggest useful therapeutic strategies that could be started in advance of any cognitive impairment in at-risk individuals.

Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, Wyss-Coray T, Mucke L, and Mahley RW: Expression of human apolipoprotein E3 or E4 in the brains of Apoe<sup>-/-</sup> mice: isoform-specific effects on neurodegeneration. J. Neurosci. 19: 4867-80 1999.

**Mutations in the APP Gene Inhibit Normal Protective Functions of the APP Protein.** In addition to plaques and tangles, another critical pathologic hallmark of the Alzheimer's disease brain is a marked loss of irreplaceable neurons. This study demonstrates that mutations of the amyloid precursor protein (APP), the cause of familial, early-onset Alzheimer's disease, impair a cell's capacity to resist a fundamental biological process (apoptosis) that culminates in cell death when cells are challenged by stressors that activate this pathway. Understanding precisely how these mutations in APP affect its normal functions will be key to protecting neurons from a premature death.

Xu X, Yang D, Wyss-Coray T, Yan J, Gan L, Sun Y, and Mucke L : Wild-type but not Alzheimer-mutant amyloid precursor protein confers resistance against p53-mediated apoptosis. Proc. Natl. Acad. Sci.USA 96: 7547-52, 1999.

**Can Tau Mutations Cause  $\beta$ -amyloid Deposition?** Frontotemporal dementia with parkinsonism, chromosome-17 type (FTDP-17) is an autosomal dominant (often familial) disease marked by behavioral and memory changes associated with shrinking of the frontal and/or temporal cortex. FTDP-17 is caused by a number of mutations in the *tau* gene. Most of these mutations affect the interaction between the microtubules of the neuronal cytoskeleton and *tau* itself. The dominant pathology in this disease is the accumulation of *tau*-containing insoluble deposits--neurofibrillary tangles--in neurons. The accumulation of  $\beta$ -amyloid-rich plaques has not been observed. The significance of this report for AD research is that it describes in a single case the presence of both diffuse and neuritic  $\beta$ -amyloid-containing plaques in a relatively young individual. The authors suggest the possibility that  $\beta$ -amyloid deposition in this case might be in response to the underlying tau pathology, although it could also be due to concurrent AD and FTDP-17 pathology. Most scientists believe that  $\beta$ -amyloid causes *tau* pathology and an example of the converse effect would be very exciting news.

D'Souza I, Poorkaj P, Hong M, Nochlin D, Lee VM, Bird TD, and Schellenberg GD: Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. Proc. Natl. Acad. Sci. USA 96: 5598-603, 1999.

**Gene Controlling Life-Span Identified.** A long sought after goal of aging research is to identify genes that control or modulate life-span. Scientists have discovered in fruit flies a gene, called *methuselah*, which when mutated extends life-span by about one-third and which provides enhanced resistance to various forms of stress, including starvation, high temperature, and tissue-damaging free radicals. The protein product of the *methuselah* gene may be a cell surface receptor that plays a central role in determining how well cells respond to stress. The better a cell is at resisting oxidative stress, for example, the longer the cell will survive. Understanding *methuselah* gene function and identifying its mammalian counterpart should enhance our knowledge of mechanisms relevant to aging, especially with respect to maintaining survival of long-lived brain cells (neurons).

LinYJ, Seroude L, and Benzer S: Extended life-span and stress resistance in the drosophila mutant Methuselah. Science 282: 943-46, 1998.

**Lorenzo's Oil Prevents Neurodegeneration in a Fly Model of Human Disease.** Fruit flies are being used to identify genetic mutations that cause nervous system dysfunction resembling that seen in human neurodegenerative conditions. One fly mutant (termed *bubblegum*) exhibits age-dependent degeneration of neurons in the eye and elevated levels of certain types of fatty acids. The mutant gene in *bubblegum* flies codes for an enzyme that breaks down fatty acids, similar to an enzyme impaired in human adrenoleukodystrophy (ALD). ALD is characterized by high levels of fatty acids (similar to those seen in *bubblegum*) and progressive degeneration of brain neurons. Feeding the *bubblegum* flies Lorenzo's oil, a mixture of unsaturated fatty acids, blocked the accumulation of fatty acids and thus stopped or prevented neurodegeneration. By using mutant flies, researchers can screen for potential ALD treatments and gain further insights into the molecular basis of ALD.

Min KT and Benzer S: Preventing neurodegeneration in the drosophila mutant bubblegum. Science 284: 1985-88, 1999.

**New Gene Causing Dementia Discovered.** Aberrant protein interactions leading to the formation of insoluble aggregates, or so-called inclusion bodies, are becoming a hallmark of many neurodegenerative disorders. Scientists have now identified a new disease, familial encephalopathy with neuroserpin inclusion bodies, characterized by dementia in mid-life and by the presence in brain tissue of unique inclusion bodies made of neuroserpin, a protein which blocks the breakdown of other proteins. This disease is caused by two mutations in the neuroserpin gene. These mutations are hypothesized to lead to the formation of abnormal neuroserpin protein, which aggregates over time and causes neuronal cell dysfunction. Finding ways to block protein aggregation may suggest new therapeutic strategies in this and other neurodegenerative disorders.

Davis RL, Shrimpton AE, Holoham PD, Bradshaw C, Feiglin D, Collins GH, Sonderegger P, Kinter J, Becker LM, Lachawan F, Krasnewich D, Muenke M, Lawrence DA, Yerby MS, Shaw CM, Gooptu B, Elliot P, Finch JT, Carrell RW, and Lomas DA: Familial dementia caused by polymerization of mutant neuroserpin. *Nature* In press.

**Mammalian Clock Genes.** Genes regulating the sleep-wake cycle are being discovered. The first mammalian Clock gene was identified and its structure was determined in 1994. Using data from the Wisconsin Sleep Cohort Study, one change in the DNA code for this gene was found to be significantly associated with preference for later sleep times, equivalent to a 10 to 44 minute difference in preferred timing. More recently, a gene for narcolepsy in dogs has been identified. This gene codes for a family of brain chemical signaling agents (neuropeptides) known as hypocretins. These neuropeptides are made only in an area of the brain that also regulates basic body functions such as feeding and energy balance, blood pressure, and central regulation of the immune system (homeostasis). The brain cells that contain them make connections with many of the brain regions involved in regulating the sleep-wake cycle. The hypocretins may act as chemical signals involved in the mechanisms of homeostasis and alertness.

Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS, and Mignot E: A *CLOCK* polymorphism associated with human diurnal preference. *Sleep* 21:569-76, 1998.

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qui X, de Jong, Nishino S, Mignot E: The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* August 6, 1999.

Peyron C, Tighe DK, Van den Pol AN, De Lecea L, Heller HC, Sutcliffe JG, and Kilduff TS: Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 18:9996-10015, 1998.

**Inhibition of Inappropriate Thoughts and Impulses.** Normal human behavior and cognition depend on the ability to suppress inappropriate thoughts, impulses, and actions. As a first step in understanding how inhibitory control may develop or go awry--as in Tourette's syndrome and obsessive-compulsive disorders--scientists at the Medical College of Wisconsin, Milwaukee, used functional magnetic resonance imaging to study event-related brain activity in 14 men and women. Their results suggest that the ability to suppress responses to irrelevant information is governed by a distributed cortical network centered in the right hemisphere of the brain.

Garavan H, Ross TJ, and Stein EA: Right hemispheric dominance of inhibitory control: An event-related functional MRI study. *Proceedings of the National Academy of Sciences USA* 96:8301-8306, 1999.

**Cytomegalovirus Accelerates AIDS Progression in Infants.** Although cytomegalovirus (CMV) has long been suspected of speeding progression to AIDS in individuals who are infected with the human immunodeficiency virus (HIV), a direct causal relationship has been difficult to prove because CMV infections are widespread among adults. But a recent multicenter study assessed the health of 440 infants born to HIV-infected women and confirmed that coinfection with HIV and CMV can significantly accelerate the development of AIDS symptoms, death, and central nervous system disease compared to infection with HIV alone. The researchers suggest

that future studies should rigorously evaluate strategies for preventing CMV infections in HIV-infected newborns and infants. [secondary B treatment]

Kovacs A, Schluchter M, Easley K, Demmler G, Shearer W, La Russa P, et al.: Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. New England Journal of Medicine 341:77-84, 1999.

### **Human Myostatin Gene Expression Contributes to Muscle Wasting in HIV-Infected Men.**

The protein myostatin is known to slow the growth of skeletal muscle in developing animal embryos, but little is known about the protein's production and function in human adults. Suspecting that myostatin might contribute to disease-associated muscle loss, scientists at the Charles R. Drew University cloned the human myostatin gene and cDNA and examined the gene's expression in blood and skeletal muscle from healthy and HIV-infected men. Because myostatin levels were elevated in HIV-infected men who had also experienced severe weight loss, the researchers concluded that myostatin attenuates growth of skeletal muscle and contributes to HIV-associated muscle wasting in adults. The findings may find therapeutic applications to the muscle-wasting conditions associated with HIV infection, cancer, and old age. [secondary B treatment]

Gonzales-Cadavid N, Taylor N, Yarasheski K, Sinha-Hikim I, Ma K, Ezzat S, Shen R, Lalani R, Asa S, Mamita M, Nair G, Arver S, and Bhasin S: Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. Proceedings of the National Academy of Sciences USA 95:14938-14943, 1998.

**The Eyes Have It.** Scientists have long believed that each eye develops independently after birth, with altered or obstructed vision in one eye having no effect on the growth and quality of vision in the other. But recent investigations suggest otherwise. By evaluating 15 monkeys reared from birth with a specially made contact lens over one eye, scientists at the Yerkes Regional Primate Research Center discovered that visual development in an untreated eye could be significantly altered by modifying vision in the other. These findings may eventually point to new avenues for treating and preventing visual defects in infants and young children. [secondary B treatment]

Bradley DV, Fernandes A, Boothe RG: The refractive development of untreated eyes of rhesus monkeys varies according to the treatment received by their fellow eyes. Vision Research 39:1749-1757, 1999.

**Effects of Low Level PCB Exposure on Male Reproduction.** Widespread contamination with polychlorinated biphenyl from the manufacture of electrical equipment poses a major health concern. A recent study of mice evaluated the effect of PCBs on several aspects of male reproduction. Data indicated that male mice exposed to a low level of PCBs during fetal development and later in their subsequent diets displayed some functionally impaired sperm and enlarged testes but no overall decrease in natural breeding ability. These results suggest that PCB exposure causes subtle changes in sperm but not overt changes in male fertility.

Huang, A, Powell D and Chou K. Pre- and postnatal exposure to 3,3', 4,4'-tetrachlorobiphenyl: I. Effects on breeding ability and sperm fertilizing ability in male mice. Archives of Environmental Contamination and Toxicology 34: 204-8, 1998.

**Chimp Origin of HIV Found.** Scientists believe they have determined the likely origin of HIV type 1 (HIV-1), the dominant form of HIV in the global epidemic, from a strain of simian immunodeficiency virus (SIV) isolated from the common chimpanzee (*Pan troglodytes troglodytes*). Based on several lines of evidence, including genetic sequence analysis and geographic coincidence, they hypothesize that the common chimpanzee is the primary carrier of HIV-1 and the source of the original introduction to humans. Studies on the biology and natural history of SIV and HIV-1 infections in chimpanzee and humans, respectively, are in progress. These studies should yield further insight on cross-species transmission of viruses from animals to humans.

Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, Sharp PM, Hahn BH: Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. Nature 397: 436-441, 1999.

**Weakened Virus Still Causes Disease in Primates.** Many licensed vaccines for diseases other than AIDS are live but attenuated (weakened) versions of the normally disease-causing virus. Since AIDS is deadly, this approach has only been tested using the primate model of HIV, called simian immunodeficiency virus (SIV). Recently, scientists have demonstrated that infants and adult macaque monkeys exposed to HIV, attenuated through deletion of portions of several genes required for viral replication, eventually show signs of the disease, raising new concerns about the safety of this approach. These results suggest that further research on live attenuated HIV vaccine candidates should focus on attenuation methods other than those based on impairment of viral replication.

Baba TW, Liska V, Khimani AH, Ray NB, Dailey PJ, Penninck D, Bronson R, Greene MF, McClure HM, Martin LN, Ruprecht RM: Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. Nature Medicine 5(2): 194-203, 1999.

**Routine Maternal Use of AZT is Safe for Children.** Since early 1994, the medical treatment of HIV-infected pregnant women in the United States includes the routine use of AZT (zidovudine) for the prevention of mother-child transmission. Several recent studies demonstrate that AZT is well tolerated by HIV infected mothers and that uninfected children treated with AZT *in utero* shows no adverse effects in the areas of growth, cognitive/developmental function, immune function, cancers, or mortality. Therefore, the recommendation to use the AZT regimen to prevent mother-child HIV-1 transmission remains appropriate.

Culnane M, Fowler MF, Lee SS, McSherry G, Brady M, O'Donnell K, Mofenson L, Gortmaker SL, Shapiro DE, Scott G, Jimenez E, Moore EC, Diaz C, Flynn PN, Cunningham B, Oleske J: Lack of long-term effects of *in utero* exposure to zidovudine among uninfected children born to HIV-infected women. Journal of the American Medical Association 281(2): 151-157, 1999.

Hanson IC, Antonelli TA, Sperling RS, Oleske JM, Cooper E, Culnane M, Fowler MG, Kalish LA, Lee SS, McSherry G, Mofenson L, Shapiro DE: Lack of Tumors in Infants with Perinatal HIV-1 Exposure and Fetal/Neonatal Exposure to Zidovudine. Journal of Acquired Immune Deficiency syndromes and Human Retrovirology 20(5): 463-467, 1999.

McSherry GD, Shapiro DE, Coombs RW, McGrath N, Frenkel LM, Britto P, Culnane M, Sperling RS: The effects of zidovudine in the subset of infants infected with human immunodeficiency virus type-1 (Pediatric AIDS Clinical Trials Group Protocol 076). Journal of Pediatrics 134(6): 385-393, 1999.

**Cellular Immunity May Not be Necessary for HIV/AIDS Vaccine.** One of the problems facing AIDS vaccine investigators has been the inability to obtain a sufficiently protective immune response, especially a cytotoxic T lymphocyte (CTL) response (also known as cellular immunity). A new study has developed an immunization approach in the monkey model that leads to the suppression of a hybrid HIV/SIV virus in the absence of CTL activity. This finding supports the concept that protection might not necessarily be determined by CTLs, and could be achieved through other unknown T cell activity.

Robinson HL, Montefiori DC, Johnson RP, Manson KH, Kalish ML, Lifson JD, Rizvi TA, Lu S, Hu S-L, Mazzara GP, Panicali DL, Herndon JG, Glickman R, Candido MA, Lydy SL, Wyand MS, McClure HM: Neutralizing antibody-independent containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations. Nature Medicine 5(5): 526-534, 1999.

**Possible Relief for Allergy Sufferers.** The symptoms of asthma and allergies are triggered by the release of histamines from cells known as mast cells. These cells possess a surface receptor molecule, called FcεRI, which is involved in mast cell activation in response to substances that cause allergies called allergens. Recently, scientists have made crystals of FcεRI and used a technique called x-ray crystallography to determine the detailed three-dimensional structure of this molecule. This is a major advance in understanding the basic mechanisms of asthma and allergic diseases, and may lead to the development of a new class of anti-allergy drugs.

Garman SC, Kinet J-P, Jardetzky TS: Crystal Structure of the human high-affinity IgE receptor. Cell 95: 951-961,

**Key Immune System Enzymes Essential to Health.** The innate or native immunity is the first line of defense against microbial infection and includes physical barriers such as mucosal membranes, several types of phagocytic (scavenger) cells, and various blood-borne molecules. It is referred to as native immunity because it does not require prior exposure to foreign substances for activation as is the case with acquired or specific immunity. By genetically engineering mice with mutations in the genes of one type of innate immune system cell, the macrophage, scientists have determined the function and importance of two innate immune mechanisms, reactive oxygen molecules produced by an enzyme called *Aphox*, and reactive nitrogen molecules produced by an enzyme called NOS2. These findings enhance our understanding of the innate immune response to microbial infections.

Shiloh MU, MacMicking JD, Nickolson S, Brause JE, Potter S, Marino M, Fang F, Dinauer M, Nathan C:

Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. Immunity 10: 29-38, 1999.

**Mast Cells Protect Against Bacterial Infection.** Mast cells are known to trigger allergy symptoms by secreting histamine and other inflammation mediators. Researchers have found that human and mouse mast cells cultured in the laboratory directly protect against deliberate infection by bacteria. This finding enhances our understanding of the role of mast cells in the response to bacterial infection and may lead to future advances in boosting the immune system response to bacterial infection.

Arock M, Ross E, Lai-Kuen R, Averlant G, Gao Z, Abraham SN: Phagocytic and tumor necrosis factor alpha response of human mast cells following exposure to gram-negative and gram-positive bacteria. Infection and Immunity 66(12): 6030-6034, 1998.

**Progress in Developing an RSV Vaccine.** The human respiratory syncytial virus (RSV) is the most significant causative agent of pediatric respiratory tract disease worldwide. To date, RSV vaccine development has been hampered by the inability to maintain RSV in tissue culture for study purposes. Recently, scientists have engineered a strain of RSV that is viable, but grows more slowly than the wild-type RSV. This attenuated version of RSV, which can be maintained in tissue culture, will facilitate further laboratory studies on RSV and may serve as a candidate for an RSV vaccine.

Teng, MN, Collins, PL: Altered growth characteristics of recombinant respiratory syncytial viruses which do not produce NS2 protein. Journal of Virology 73(1): 466-473, 1999.

**Powerful Skin Toxin Identified.** *Mycobacterium ulcerans* is the causative agent of Buruli ulcer, a severe human skin disease primarily found in Africa and Australia. Scientists have now isolated a toxin produced by *M. ulcerans* that, when injected into guinea pigs, produces lesions similar to that of Buruli ulcer in humans. The isolation of this toxin may lead to the development of treatments and ultimately a vaccine for this disease. In addition, it will advance the current understanding of other mycobacterial diseases, such as leprosy and tuberculosis.

George KM, Chatterjee D, Gunawardana G, Welty D, Hayman J, Lee R, Small PLC: Mycolactone: A polyketide toxin from *mycobacterium ulcerans* required for virulence. Science 283: 854-857, 1999.

**Protein Signals May Be Linked to Leukemia.** Chronic myelogenous leukemia (CML) is a human leukemia associated with a cancer-causing gene, or oncogene, called Bcr-Abl. Investigators have elucidated the complicated cell signaling pathway that leads to the activation of Bcr-Abl, and ultimately the development of this form of leukemia. Another cell signaling protein, called Germinal Center Kinase Related protein or GCKR<sub>2</sub>, is active in CML cells and is associated with Bcr-Abl. Investigators demonstrated that the inhibition of this enzyme impairs Bcr-Abl-induced activation pathway. This finding strongly suggests that the GCKR enzyme is a

mediator of Bcr-Abl transformation of healthy cells to cancer cells and thus may serve as a potential target for blocking Bcr-Abl mediated CML

Shi C-S, Tuscano JM, Witte ON, Kehrl JH: GCKR links the *Bcr-Abl* oncogene and Ras to the stress-activated protein kinase pathway. Blood 93(4): 1338-1345, 1999.

**Isolation of a Potential Activator of Latent Herpes Simplex Virus.** After initial infection, herpes simplex virus (HSV) remains in cells of the nervous system in a latent state. Recently, scientists have demonstrated in a mouse model that a cellular protein, called C1 factor, may serve as a critical factor in the transition of HSV into a lytic (active) state. The study suggests that C1 factor may be an important target for new antiviral therapies.

Kristie TM, Vogel JL, Sears AE: Nuclear localization of the C1 factor (host cell factor) in sensory neurons correlates with reactivation of herpes simplex virus from latency. Proceedings of the National Academy of Sciences of the United States of America 96(4): 1229-1233, 1999.

**Sequence of Chromosome 1 of *Leishmania major*.** The *Leishmania* parasite causes a spectrum of diseases ranging from mild skin ulcers to disfiguring or lethal results. Recently, scientists have determined the genetic sequence of chromosome 1 of the *Leishmania major* genome and have identified 79 genes encoded by this chromosome. This information will help investigators understand the pathology of Leishmania and develop more specific targets for drug therapies and possibly also lead to a safe and effective vaccine for treatment and prevention of the entire range of Leishmaniasis-related diseases.

Myler P J, Audleman L, DeVos T, Hixon G, Kiser P, Lemley C, Mangess C, Rickel E, Sisk E, Sunkin S, Swartzell S, Westlake T, Bastien P, Fu G, Ivens A, Stuart K: *Leishmania major* Friedlin chromosome 1 has an unusual distribution of protein-coding genes. Proceedings of the National Academy of Sciences 96: 2902-2906, 1999.

**Potential Candidate for Herpes Simplex Virus Vaccine.** Development of a safe, effective herpes simplex virus (HSV) vaccine has been a research priority for many years. Until now, experimental HSV vaccines have been shown to be either safe but only effective in animal models, or more efficacious but potentially hazardous because of the threat of latent infection in human subjects. Investigators have constructed a double mutant HSV that can induce protective immunity in a mouse model, but that is defective in both productive and latent infection. This study provides proof of concept for the development of a vaccine for HSV as well as other viral diseases.

Da Costa X J, Jones CA, Knipe DM: Immunization against genital herpes with a vaccine virus that has defects in productive and latent infection. Proceedings of the National Academy of Science 96: 6994-6998, 1999.

**Disease Promoting Enzyme Targeted.** The enzyme known as DNA adenine methylase, or ADam®, is pivotal to the reproduction and severity of many gut-colonizing bacteria, such as

*Salmonella*, that cause food borne disease. Investigators have found that while *Salmonella* lacking Dam were able to invade mucous membranes, they showed severe defects in attacking deeper tissues. Because many bacteria, such as cholera and syphilis, have high concentrations of Dam enzymes, Dams may make excellent targets for both preventive vaccines and for therapeutic agents.

Heithoff D.M, Sinsheimer RL, Low DA, Mahan MJ: An essential role for DNA adenine methylation in bacterial virulence. Science 284: 967-970, 1999.

**Infection May Be Risk Factor for Cardiovascular Diseases.** Investigators believe a new potential risk factor for cardiovascular diseases (CVD) may be infection, particularly the acute respiratory pathogen known as *Chlamydia pneumoniae*. Atherosclerosis, a common CVD, is a chronic, inflammatory disease with lesions containing macrophage foam cells (specialized white blood cells to fight invaders). New evidence indicates that *C. pneumoniae* can induce foam cell formation, suggesting that the infection contributes to atherosclerosis. The growing evidence that infectious agents play a role in the development of CVD may lead to preventive approaches using antibiotics.

Kalayoglu MV, Byrne GI: A *Chlamydia pneumoniae* component that induces macrophage foam cell formation is chlamydial lipopolysaccharide. Infection and Immunity 66(11): 5067-5072, 1998.

**Psychosocial Implications of XSCID.** X-linked severe combined immunodeficiency (XSCID) is the rare "Bubble boy" disease, a complete lack of a functional immune system with consequent susceptibility to life-threatening infections. Affected infants are males due to the X chromosome location of the disease gene; they must currently receive bone marrow transplants to survive. Female relatives may carry the gene defect and pass it on to male offspring even though they themselves are healthy. Researchers are studying (i) whether healthy siblings of XSCID males share the recognized psychological features of siblings of persons other genetic diseases such as cystic fibrosis, and (ii) whether distinct features might be particularly characteristic of families with a rare, X-linked disease with severe infantile presentation, but potential medical treatment. One consistent emerging theme is a lack of guilt feelings experienced by those who do not inherit the family's disease mutation (survival guilt). (This contrasts with siblings in families with cystic fibrosis or hereditary breast cancer.) Sisters of males with XSCID frequently express desire to bear healthy male children as a "gift" to their mothers, whose sons suffered and in many cases died of XSCID. Family stresses are great due to long hospitalization of affected infant sons. Of great practical importance is the discovery of persistent misunderstandings of the disease by siblings who at a very young age donated bone marrow to XSCID-affected baby brothers. These results are adding new knowledge about psychological aspects of genetic disorders and their impact on family members. Different modes of inheritance and disease manifestations may produce distinct sets of psychological difficulties.

Puck J, Fanos F, Davis J: Sibling knowledge and attitudes toward carrier testing for X-linked severe combined immunodeficiency. American Journal of Human Genetics (in press, 1999).

**Isolation and Characterization of Human Cementoblasts.** Cementum is a unique mineralized tissue that plays a critical role in oral physiology by providing an appropriate surface for anchoring teeth within bone. However, the biochemical properties of the cells that produce this structure have remained elusive due to a lack of appropriate cell culture model systems. Using a new approach, human cementoblasts were isolated for the first time, and found to be similar, but not identical, to cells that produce bone. This provides an excellent model system to study the physiology of this unusual cell type, and for future design of periodontal reconstructions.

Grzesik WJ, Kuznetsov SA, Uzawa K, Mankani M, Gehron Robey P, and Yamauchi M: Normal human cementum-derived cells: Isolation, clonal expansion and *in vitro* and *in vivo* characterization. J Bone Mineral Res 13: 1547-54, 1998.

Grzesik WJ, Cheng H, Oh JS, Kuznetsov SA, Mankani MH, Uzawa K, and Gehron Robey P: Cementum-forming cells are phenotypically distinct from bone-forming cells. J Bone Mineral Res in press, 1999.

**Mutations in GNAS1 Cause Fibrous Dysplasia of Bone.** Fibrous dysplasia of bone (FD) is a crippling skeletal disorder which has serious consequences including fractures, impairment of limb function, facial and limb deformities and compression damage to sensory nerves that can result in blindness or deafness. It can involve single or multiple bone lesions and is often found in association with the McCune-Albright syndrome, which is known to arise from a post-zygotic mutation in the GNAS1 gene. An in depth histological study of FD lesions revealed substantial variation in the lesions, and all patients were found to have mutations of the GNAS1 gene. These results indicate that GNAS1 mutations result in a broad spectrum of bone lesions beyond the McCune-Albright syndrome, and suggest that new therapies may have broader impact than previously realized.

Bianco P, Riminucci M, Majolagbe A, Kuznetsov SA, Collins MT, Mankani MH, Corsi A, Bone HG, Weintraub S, Spiegel AM, Fisher LW, and Gehron Robey P: Activating mutations of the GNAS1 gene, stromal cell dysfunction, and osteomalacic changes in non-McCune-Albright fibrous dysplasia of bone. J Bone Mineral Res in press, 2000.

Riminucci M, Liu B, Corsi A, Shenker A, Spiegel AM, Gehron Robey P, and Bianco P: The histopathology of fibrous dysplasia of bone in patients with activating mutations of the Gs alpha gene: Site specific patterns and recurrent histological hallmarks. J Pathol 187:249-58, 1999.

Riminucci M, Fisher LW, Majolagbe A, Corsi A, Lala R, DeSanctis C, Gehron Robey P, and Bianco P: A novel GNAS1 mutation, R201G, in McCune-Albright Syndrome (MAS). J Bone Mineral Res in press, 1999.

**Histatins B Promising Antifungal Agents.** *Candida albicans* is the predominant species of yeast isolated from patients with oral candidiasis, which is frequently a symptom of human immunodeficiency virus infection and is a criterion for staging and progression of AIDS. Salivary histatins (Hsts) are potent *in vitro* antifungal agents and have great promise as therapeutic agents in humans with oral candidiasis. The histatins bind to specific receptors on the fungal membranes. Hst-5 appears to be the most potent of the salivary Hsts. NIH-funded investigators are studying the mechanism of Hst-5 yeast killing. [secondary B treatment]

Koshlukova SE, Lloyd TL, Araujo MWB, and Edgerton M: Salivary histatin 5 induces non-lytic release of ATP from *Candida albicans* leading to cell death. J Biol Chem 274: 18872-79, 1999.

**New Insights into Cartilage Development.** Cartilage has a unique extracellular matrix structure specifically designed to resist compression in synovial joints, and also provides the template for bones during embryonic development. Link protein is one of the major cartilage matrix proteins, but its specific function is not clear. Recently, by generating a mouse model deficient in link protein, scientists identified a critical role for link protein in the maintenance of cartilage structure and function during skeletal development. The phenotype of link protein-deficient mice should provide useful clues for the identification of gene defects in human chondrodysplasias.

Watanabe H and Yamada Y: Mice lacking link protein develop dwarfism and craniofacial abnormalities. Nature Genetics 21: 225-29, 1999.

**Molecular Mechanism for Cleft Palate.** An international collaborative study, supported in part by the NIH, has identified a molecular pathway responsible for cleft palate in an animal model. The investigators used a "knock-out" mouse model to establish a link between cleft palate and a molecular cascade involving transdermal growth factor  $\alpha$  (TGF- $\alpha$ ), epidermal growth factor receptor (EGFR), and a group of protein-digesting enzymes known as matrix metalloproteinases (MMPs). The study identified a molecular explanation for a very complex birth defect and points the way to identifying other genetic and environmental contributing factors, as well as potential interventions.

Miettinen PJ, Chin JR, Shum L, Slavkin HC, Shuler CF, Derynck R, and Werb Z: Epidermal growth factor receptor function is necessary for normal craniofacial development and palate closure. Nature Genetics 22: 69-73, 1999.

**Candidate Taste Genes Identified.** A collaborative effort between intramural and extramural scientists identified the genes likely responsible for the mammalian sense of taste. The researchers identified genes that encode two novel proteins expressed in cells specifically geared to the sense of taste. The isolation of the candidate taste receptor genes provides the groundwork necessary for manipulating the perception of taste and stimulating or blocking taste cell function. The discovery could one day hold implications for engineering foods and medicines to specific taste qualities.

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJP, and Zuker CS: Putative mammalian taste receptors: a class of taste specific GPCRs with distinct topographic selectivity. Cell 96: 541-51, 1999.

**Cartilage to Bone: The Role of Vascular Endothelial Growth Factor (VEGF).** Long bones grow as expanding growth plate cartilage is replaced by bone through the process known as endochondral ossification. Precise coupling of both cartilage production (chondrogenesis) and bone formation (osteogenesis) is critical. Blood vessel invasion of cartilage (angiogenesis) was

known to be an important initial step, but little was known about factors regulating that step. A recent study has demonstrated that vascular endothelial growth factor, or VEGF, is a regulator of blood vessel formation during bone development, and that blood vessel invasion of cartilage is a necessary component of proper bone formation. These findings may lead to improved understanding of some diseases characterized by skeletal growth impairments.

Gerber H-P, Vu TH, Ryan AM, Kowalski J, Werb Z, and Ferrara N: VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nature Medicine* 5: 623-28, 1999.

**Degradation of Oxidatively Damaged Histones Occurs Through Poly-ADP Ribose-activated 20S Proteasome.** Oxidative stress leads to degradation of a large number of oxidized and damaged proteins. This study shows that activation of the 20S proteasome, following oxidative stress, is dependent upon poly-ADP ribosylation. It is the nuclear form of this protein degradation complex that removes oxidatively damaged histones.

**Novel Ubiquitin Chains in DNA Repair.** Proteins marked with ubiquitin are targeted for proteolysis. This study has revealed that a novel ubiquitin-conjugating enzymes functions during postreplicative DNA repair to provide proper signaling functions.

**Apoptosis in the Absence of Caspase Activity.** The degradation of proteins by enzymes known as caspases has been shown to play a predominate role during programmed cell death, also known as apoptosis. Recently, scientists have shown that various characteristics of apoptosis including the loss of cell volume and intracellular potassium, changes in the mitochondrial membrane potential, and the loss of cell viability can occur in the absence of caspase activity. Studies show that this caspase-independent cell death occurs under conditions when a non-membrane bound receptor is used to signal apoptosis. Interestingly, internucleosomal DNA degradation, which is a classic feature of apoptosis, is inhibited under all cell death conditions, suggesting that this characteristic of programmed cell death is caspase-dependent. Additionally, it was determined that changes in the mitochondrial membrane potential are restricted to the shrunken population of cells. Together these data suggest that cell shrinkage, potassium efflux, and changes in the mitochondrial membrane potential are tightly coupled, but occur independent of DNA degradation, and can be largely caspase-independent depending on the particular signal transduction pathway.

**Report on the Health Effects of Electrical and Magnetic Fields.** Electric and magnetic fields (EMF) are associated with the production, transmission, and use of electricity; thus, the potential for human exposure is high. As part of an 1992 Energy Policy Act, Congress requested that the NIH investigate whether exposure to these electric and magnetic fields had an adverse effect. As the result of a 6 year program combining original studies with a comprehensive review of the existing knowledge in the field, it was concluded that "The scientific evidence suggesting that extremely low frequency-electric and magnetic fields (ELF-EMF)] pose any health risk is weak.

This report also concludes that ELF-EMF exposure cannot be recognized as entirely safe because of weak scientific evidence that exposure may pose a leukemia hazard." The latter conclusion that exposure cannot be recognized as entirely safe was based on epidemiology studies. In contrast, studies in cells and animals failed to demonstrate any consistent pattern across studies although sporadic findings of biological effects have been reported. For example, when the ability of 50- and 60-Hz magnetic fields to promote mammary gland tumors initiated by the administration of 7,12-dimethylbenz-(a)anthracene (DMBA) was examined in female rats in 13- and 26-week whole-body exposure studies, no evidence that magnetic fields promoted the development of mammary gland neoplasms was found. Similarly, in 2-year whole-body exposure studies, there was only equivocal evidence of carcinogenic activity of 60-Hz magnetic fields in male F344/N rats based on increased incidences of thyroid gland C-cell neoplasms in some groups. There was no evidence of carcinogenic activity in female F344/N rats or male or female B6C3F1 mice exposed to 0.02, 2, or 10 G, or 10 G intermittent 60-Hz magnetic fields at sites for which epidemiology studies have suggested association with magnetic fields (brain, mammary gland, leukemia).

**Cloning of a Novel Kidney Cytochrome P450 Enzyme that Metabolizes Fatty Acids.** NIH scientists have reported the cDNA cloning and heterologous expression of CYP2J5, a new mouse P450 that is abundant in the kidney and active in the biosynthesis of fatty acid epoxides and alcohols. CYP2J5 mRNA and CYP2J protein(s) are primarily localized to regions of the nephron where CYP2J5 products have direct effects on fluid/electrolyte transport and are known to mediate the actions of hormones such as angiotensin II. CYP2J5 is an enzymologically distinct, developmentally regulated, protein that is highly localized to specific nephron segments and contributes to the oxidation of endogenous renal arachidonic acid pools. It is postulated that CYP2J5 products play important functional roles within the kidney.



## **STORIES OF DISCOVERY**

### **Drug Exposed Children: What the Science Shows**

Highly emotional television and magazine reports in the mid eighties captured the public's attention with horror stories about crack babies.<sup>6</sup> Wild speculation, based on little scientific evidence, about the health, educational and societal costs associated with infants who were exposed to cocaine before birth abounded. A positive note in this rush to judgment, however, was the substantive effort on the part of the scientific community to understand the effects that prenatal drug exposure had on the developing child.

Although this particular story begins with the scientific strides that have been made since a New England Journal of Medicine article suggested in 1985 that cocaine influences the outcome of pregnancy as well as the neurologic behavior of newborns, concern about the passage of drugs through the human placenta and through the breast milk of the drug using mother dates back to the end of the 19th century.

In the case of opiates, the occurrence of newborn drug withdrawal associated with maternal opiate dependency has been known for over a century, but it was the reemergence of heroin use in the late 1950s and early 1960s among women of childbearing age that generated concern about the effects of opiates on the developing fetus. The advent of methadone maintenance for pregnant opiate-dependent women in the early 1970s provided the impetus for investigations to determine both short- and long-term neurobehavioral effects of prenatal opiate exposure. Studies comparing infants born to heroin-addicted women not maintained on methadone with infants born to heroin-addicted women receiving methadone have found higher birthweights in infants born to the methadone maintained women. Overall, the research done on the effects of prenatal opiate exposure indicates that opiate exposed infants through 2 years of age function well within the normal range of development and that children between 2 and 5 years of age do not differ in cognitive function from children born to nondrug-dependent women from comparable socioeconomic and racial backgrounds. When research began to show dramatic increases in cocaine use among women of child bearing age concern shifted to maternal cocaine use.

Today in the 1990s, NIH estimates that 5.5% or 221,000 women use some illicit drug during pregnancy, thus 221,000 babies are potentially born drug-exposed (National Pregnancy and Health Survey, 1992). The full extent of the effects of prenatal drug exposure on a child is still not completely known, but science has shown that babies born to mothers who abuse drugs during pregnancy are often prematurely delivered, have low birth weights, smaller head circumferences, and are often shorter in length. Estimating the full extent of the consequences of maternal drug abuse is more difficult, and determining the specific hazard of a particular drug to the unborn child is even more problematic given that more than one substance is typically abused. Factors such as the amount and number of all drugs abused, inadequate prenatal care, socio-economic status, poor maternal nutrition, other health problems, and exposure to sexually transmitted diseases are just some examples of why it is difficult to determine the direct impact of perinatal cocaine use, for example, on maternal and fetal outcome. This is one of the reasons

why research in this area also relies heavily on animal models. Many of the animal models show behavioral changes similar to those being found in children, but many also find specific and permanent changes in the animal's brain.

A recent animal study has demonstrated the presence of cocaine receptors in the brains of fetal rats and showed that cocaine is able to bind directly to these brain sites, which could, then, be a mechanism to modify brain development and later behavior, by modifying brain activity directly. With this new information about cocaine receptors in fetal brain tissue, researchers may be able to pinpoint the specific sites in the developing brain that are most vulnerable to cocaine and develop better prevention and treatment strategies.

In addition to animal findings, there has been a significant amount of research conducted in humans. For example, there are quite a number of studies that are following a large number of drug-exposed children through their school years. Researchers who have been studying many of these children since birth are looking not only at the child's intellectual status, but at their behavioral, emotional, and social development as well. Using sophisticated technologies, scientists are finding that exposure to cocaine during fetal development may lead to subtle, but significant deficits later on, especially with behaviors that are crucial to success in the classroom, such as blocking out distractions and concentrating for long periods of time.

For example, researchers who have been following a group of nearly 500 children for six years have found that many cocaine-exposed, four-and-a-half year olds are more impulsive and more easily distracted than their peers. Also, researchers reported on the development, at six years of age, of children who have been studied since the prenatal period. The children prenatally exposed to cocaine showed deficits in their ability to sustain attention on a computerized vigilance task when they were tested and compared to their non-drug-exposed peers. Other studies are also showing subtle cognitive and learning problems in some prenatally cocaine exposed children appearing as they enter middle school. Because these effects can be subtle and only expressed as children develop, long-term follow-up is needed. Long term or longitudinal studies will also enable us to examine whether prenatally drug-exposed children are themselves more vulnerable, or at increased risk for drug abuse in childhood and adolescence. Given the importance of environmental factors on child development, these researchers are also examining critical factors such as parenting and socioeconomic conditions.

Although much has been learned about the effects that prenatal drug exposure does and does not have on children, much more well-documented longitudinal research remains to be done if science is to replace popular ideology on this topic.

## **Neurobiology of Addiction: The Role of Dopamine**

Cocaine, methamphetamine, heroin, marijuana and nicotine are five very powerful addictive substances that appear to have even more in common than the heavy health, social and financial burden they place on individual users, their families, and society. Recent evidence suggests that major aspects of their addictive properties may well stem from their similar effects on a common neurochemical system. Scientists have been studying the mechanisms of how drugs such as these exert their powerful effects on the brain and the body since the late 19th century. In fact, studies of drug action have revolutionized our overall understanding of how the brain works. Pharmacological studies of nicotine, initiated in 1889, eventually led to the concept of neurotransmitters and receptors that was formulated in the early 20th century. The first neurotransmitter to be identified was acetylcholine in the 1920s. A key piece of information that led to this discovery was the finding that nicotine mimics acetylcholine at the nerve muscle junction. This discovery was the basis for the theory on how other drugs such as cocaine, heroin, and marijuana must work, by mimicking natural substances in the body, such as neurotransmitters.

The identification of additional neurotransmitters took decades to achieve in large part because of the limited technology available to study the brain's complexity. By the 1950's, researchers had begun to develop the sensitive tools needed to distinguish and measure the small amounts of neurotransmitters present in the brain. Of particular interest at that time were the compounds dopamine, serotonin, and norepinephrine. Stimulant drugs such as cocaine and amphetamine were shown to increase the amounts of these transmitters in the brain and, thus, it was hypothesized, produce the behavioral effects seen after use of these drugs. By the early 1960's evidence began to emerge that linked dopamine to Parkinson's disease as well as to psychiatric disorders such as schizophrenia. Intertwined with these findings were the realizations that cocaine and amphetamine could cause schizophrenia-like psychoses and could selectively impact dopamine neural communication. These early observations fueled scientific interest in dopamine, its function, location, and its role in behavior, psychiatric disorders, and drug response and addiction.

As technology has rapidly advanced in recent years, so has our knowledge of dopamine's role in brain function. Dopamine containing neurons are relatively rare in the brain (tens of thousands of neurons out of approximately 100 billion). They are clustered in key neural circuits that are involved in regulating movement as well as feelings of pleasure in what is commonly referred to as the reward circuit. Factors that impact the concentration of dopamine in these circuits have been shown to have profound effects on the individual. For example, too little dopamine can trigger tremors and paralysis like that observed in Parkinson's disease. Too much dopamine can result in the hallucinations of schizophrenia and behavioral activation in drug abusers.

As scientists explore the intricacies of the dopamine system, a greater understanding of drug addiction continues to unfold. A convergence of data now points toward one commonality among many very different drugs such as cocaine, methamphetamine, heroin, nicotine, and marijuana: they all can elevate levels of the neurotransmitter dopamine. It is this effect on the

dopamine system, either directly or indirectly, that is thought to contribute to the addictive nature of these substances.

Recently a team of researchers has published the strongest evidence to date that the surge of dopamine in addicts' brains is what triggers a cocaine high. Using brain imaging technology, scientists were able to track the rise of dopamine and link it to feelings of euphoria in cocaine addicts. Another team of researchers has shown the unique temporal and regional patterns of brain activity associated with cocaine euphoria and craving. These studies are confirming what had been shown in exquisite detail in earlier animal studies, that brain regions containing dopamine, such as the nucleus accumbens, are central to cocaine's addictive effects.

While cocaine and the amphetamines act directly on dopaminergic neurons, evidence is emerging that other drugs such as opiates, nicotine, and marijuana can alter dopamine levels indirectly, through a cascade of neuronal activity. In the mid-1980's, it was shown that nicotine could elicit dopamine release in the nucleus accumbens of rats. In 1997, this same group produced a more detailed mapping of the dopaminergic response to nicotine administration in the rat, showing that the outermost portion of the nucleus accumbens, the shell, was the most affected. This followed on the heels of earlier papers showing that cocaine, amphetamine, and morphine can also provoke a selective release of dopamine in this same region. In June 1997, it was further confirmed that  $\Delta^9$ -tetrahydrocannabinol (THC), the active ingredient of marijuana, given to rats, could also increase dopamine levels in the nucleus accumbens.

The recognition that dopamine is a key and common end point in addiction opens up new avenues of drug treatment.

## The Link Between Oral Biofilm Infections and Systemic Disease

Bacteria are remarkably adept at surviving feast and famine, capable of adjusting their needs to accommodate highly diverse environments. Scientific inquiry has discovered a number of microbial characteristics that facilitate adaptations to changing environments. Among them is the capacity to form and maintain biofilms. Many of us have had the experience while hiking to have slipped or fallen on wet rocks near a stream. This is not only because water tumbles over these rocks and makes them hazardous, but also because colonies of slippery, slimy creatures often will have made such rock surfaces their home. Among the culprits most likely to fell a hiker is something called "biofilm", a community of many species of microorganisms.

One of the first biofilms linked to human diseases was dental plaque B a sticky film of bacteria that coats the surfaces of the teeth and gums. In the 17th century, Antoni van Leeuwenhoek first observed "animalcules" swarming in the tartar on his teeth and, even after meticulous cleansing, deposits remaining between his teeth were as thick as Abatter@. These deposits, he observed, contained a mat of various forms of bacteria. As early as the 1960s, dental scientists implicated the bacteria in dental plaque in the most prevalent of oral diseases, dental caries and periodontal diseases. In the 1970s, biofilms were detected on a heart pacemaker and in urinary catheters. A biofilm microbe, *Legionella pneumophila*, was the source of the *Legionnaire's disease outbreak that killed 29 in Philadelphia in 1976*. The 1980s brought confirmation of the existence of biofilms in the lungs of cystic fibrosis patients. Today, there is evidence that biofilms are involved in a wide variety of human infections including dental caries, periodontitis, biliary tract infection, otitis media, musculoskeletal infections, bacterial prostatitis, native valve endocarditis, cystic fibrosis pneumonia, and many different nosocomial or hospital-acquired infections. Bacteria in biofilms are less susceptible to both host immune responses and antibiotics.

The adult human body consists of  $10^{15}$  somatic cells and  $10^{14}$  normal or commensal microbes. Commensal bacteria reside within complex biofilm ecosystems that are found on the surfaces of teeth and/or prosthetic implants, and the surfaces of the mucosal epithelia that line the oral cavity, respiratory tract, esophagus, gastrointestinal tract, and urinary tract. Under a variety of conditions, some of these microorganisms become opportunistic and are associated with local or systemic infections such as Hemophilus influenza, Streptococcus pneumonia, Neisseria meningitis, and Staphylococcus aureus.

**The oral cavity contains nearly half of the commensal bacteria in the human body; approximately 6 billion microbes representing 300 to 500 species. The oral microbial ecosystem is remarkably dynamic. Oral microbes can be transmitted from person to person, such as, from mother or caretaker to child, or from spouse to spouse; or can be acquired from the environment. The oral microbial ecology is extremely sensitive to potential insults confronting human hosts throughout their lifespan. From fetal life through senescence, the oral cavity is continuously challenged by opportunistic infections on the one hand and the oral complications of systemic diseases and disorders on the other. These dynamic interactions between hosts and pathogens are central to a paradigm shift in oral medicine. There is growing evidence that oral bacteria contribute to systemic disease.**

Today, we know that oral infections are associated with a number of systemic diseases and disorders. Pregnant women with oral infections have been found to have a substantially increased risk of giving birth to low-birth-weight, premature babies. People with certain heart problems or coagulation abnormalities and those with artificial joints are thought to be particularly vulnerable to some of the microbes that live in the oral cavity. Such people are often advised to take antibiotics before undergoing dental procedures that might cause bleeding and possible transient bacteremia. Diseases such as diabetes are well-documented as affecting the pathogenesis of periodontal disease; indeed, periodontal disease also affects the status of diabetes.

The number of reports associating oral infections with systemic disease has been increasing in the last few years. Most of the reports are based on epidemiological studies. A major confounding issue is that oral infections are often only one of the many important factors that can influence systemic diseases. Consequently, it is difficult to achieve the time-honored proof of relationship by cause and effect.

However, the expanded scope of recent epidemiological studies has increased the knowledge base supporting the hypothesis that commensal microbes residing in the mouth can cause an array of systemic diseases if they move into the bloodstream and thus cause bacteremia. Transient bacteremia can occur for years in patients with chronic oral infections such as periodontal disease. Bacteria have been found in the blood after tooth-brushing (40 percent of subjects), tooth extraction (60 percent) and periodontal surgery (88 percent). The hypothesis is further supported by the statistical associations between periodontal diseases and systemic diseases, which identify periodontal diseases as risk factors in that relationship.

Research on biofilms, microbial ecology and the association of oral biofilm infections with systemic diseases is receiving increased interest. The NIH is currently sponsoring initiatives in these fascinating areas with basic, translational and clinical research studies on these relationships in progress today. In the near future, clinical intervention studies will be supported to determine whether management of oral infections does, in fact, reduce systemic diseases and conditions.

## **Friedreich's Ataxia and Molecular Mechanisms of Iron Transport**

Iron is indispensable for the function of hemoglobin, for DNA synthesis and for a host of other life processes. Since both iron excess and iron deficiency can damage cells and organs, iron uptake, utilization, and storage are closely regulated. However, the question of how iron actually passes through cellular membranes and how its level within cellular compartments is determined remains incompletely understood. In the past three years, major breakthroughs have occurred in identifying critical genes and transport proteins involved in iron metabolism. Iron is important from a health standpoint in a number of diseases including iron deficiency and conditions characterized by iron overload, such as hemochromatosis and Cooley's anemia. Excess iron has been implicated as a risk factor for heart disease.

A major, new and previously unexpected advance is the discovery that Friedreich's ataxia is also a disorder of iron metabolism. Friedreich's ataxia (FDRA) is a debilitating neurological disease that causes degenerative changes in the spinal cord and the cerebellum. It is a genetic disease, inherited as an autosomal recessive, and strikes about one in every 50,000 persons.<sup>1,2</sup> FDRA patients rarely survive beyond early adulthood. Symptoms develop between the ages of five and fifteen years and worsen with age. Individuals with FDRA sometimes become blind and deaf, and often develop diabetes and heart failure, which is the major cause of premature death. FDRA particularly affects cells and tissues that are rapid energy metabolizers.

The first clue to understanding the cellular defects that cause FDRA came in 1996 when a gene of unknown function, named frataxin, was found to be defective in FDRA.<sup>3</sup> The gene was identified to be the site of mutations in FDRA patients, but the function of its protein product and how mutations led to disease was not understood.

Simultaneously, another team of investigators was studying iron metabolism in mitochondria in yeast. Mitochondria are discrete components of cells that provide energy--the so-called power plants of cells. This NIH-funded investigative team constructed a series of strains of yeast in which mitochondrial iron transport was altered. In the process of these experiments, they found a new gene that codes for a mitochondrial protein that is that is responsible for the transport of iron from within the mitochondria to the inner space of the cell.<sup>4</sup> To their surprise, the protein coding for this gene was virtually identical to the human protein, frataxin.

Until that time, the mechanism of damage to the mitochondria and cells in FDRA patients had been a mystery. These findings led to a new hypothesis. The researchers theorized that since the yeast and human proteins are similar in sequence and shape, they may have a similar function within the cell. They proposed that the nerve degeneration and the heart disease that characterize FDRA could be due to iron-induced mitochondrial toxicity.

This important hypothesis has generated substantial new investigation. Recent evidence for increased mitochondrial iron in FDRA patients has supported one contention of the proposal, but still left unresolved the question of whether mitochondrial defects in patients with FDRA are a direct result of iron accumulation in mitochondria, or whether the iron accumulation is secondary

result of mitochondrial damage. Frataxin-deficient mice are being developed, providing a model for thorough testing of the fundamental hypothesis for Friedreich's ataxia.

Studies have further explored the role of frataxin in mitochondrial iron transport. Deleting the frataxin gene from yeast caused mitochondrial damage that was found to be proportional to the concentration and duration of exposure to extracellular iron.<sup>5</sup> This established that the absence of frataxin can lead to excess mitochondrial iron accumulation, structural and functional damage to the mitochondria, and ultimately cell death. Reintroducing the frataxin gene resulted in the rapid export of accumulated mitochondrial iron into the inner space of the cell and restoration of function. These results establish a mitochondrial iron cycle in which the frataxin gene regulates mitochondrial iron exiting (efflux). Frataxin provides the mechanism for regulating mitochondrial iron accumulation in response to iron need, either for mitochondrial proteins or for heme synthesis, the final step of which occurs within the mitochondrial network of structures (matrix). Heme is integral to the production of hemoglobin, the oxygen-carrying protein in red blood cells.

The studies in yeast have yielded major insight into a devastating human disease, illustrating the broad ramifications that fundamental investigation in simple systems can have. These findings have offered hope to patients with this lethal disease for which at present there is no effective treatment. FDRA patients currently are being enrolled in a clinical trial designed to determine whether iron chelation therapy can remove the excess mitochondrial iron, and thereby reversing the disease process.

These findings have also greatly enhanced our understanding at the molecular level of the mechanisms of iron transport and the cellular regulation of iron metabolism. It is anticipated that they will lead to therapeutic strategies to manipulate iron uptake and iron concentrations in cellular components and to interventions of potential relevance to many diseases in addition to Friedreich's ataxia, such as hemochromatosis and other iron overload states. These findings also underscore that understanding of iron chelation needs to be improved to enhance the chelating activity and reduce the toxicity and to target specific iron pools.

## Challenging Obesity

With the approach of the 21st century, obesity emerges as one of the greatest threats to human health and well-being, increasing the risk of coronary heart disease, diabetes, stroke and some forms of cancer. A threatened epidemic of global scale, obesity has been increasing in all segments of the population at an alarming rate since 1980 with no signs of abating. In fact, in the U.S. it is now estimated that about one-quarter of the adult population is obese, defined as having a body mass index (BMI) greater than 30, and the prevalence in children has risen by 40% over the last 16 years. While the causes for this dramatic increase in the prevalence of obesity are unclear, it is likely that a changing environment, with plentiful food and little physical activity interact with a genetic susceptibility for weight gain to promote its development in many people. A staggering annual cost associated with obesity of close to \$100 billion highlights its overall impact in terms of multiple effects on health and productivity.

Though for many years evidence has demonstrated the importance of genetics in the body weight of livestock and laboratory rodents, its role in human obesity is a relatively recent development. Once adoption and twin studies provided clear evidence of a genetic component in human obesity, the emphasis shifted from *whether* to *which* specific genes are responsible for the estimated 40 to 70 percent heritable variation in obesity within a population. Over the last six years, the cloning and characterization of several mouse obesity genes as well as their human homologues has presented opportunities to identify new cellular targets for novel therapeutics.

Research took a quantum leap toward beginning to unveil the molecular underpinnings of obesity with the discovery of the obesity (*ob*) gene and the gene's product leptin. We now know that leptin occupies a prominent role in the regulation of energy metabolism through its ability to influence a complex system of signals within the brain and between the brain and the body. Primarily produced by fat cells, leptin signals the brain information about the amount of fat tissue present in the body. While few obese humans have been identified with a severe leptin deficiency, researchers have reported new insights from studies of weight gain in Pima Indians, who have a high prevalence of obesity and diabetes. After measuring leptin levels and weights of Pima subjects at the outset of one study and then tracking weight gain over a three year period, investigators found that individuals who gained weight during the study had lower initial leptin levels compared to initial leptin levels of individuals with the same starting weight who did not gain. We can conclude then that the presence of relatively low leptin levels may be a predisposition in individuals of this population to become obese. Leptin also has an effect on the efficiency of energy utilization. Humans, when placed on diets to lose weight, experience a dramatic decrease in plasma leptin levels. Identifying the receptor for leptin in mice and rats, which seems to be identical with a gene associated with gross obesity and diabetes in rodents, supplies another important piece of the leptin puzzle. The human leptin receptor gene has also been identified, providing another key to the causes and eventual treatment of obesity.

Leptin's discovery led to an explosion of research on the pathways that regulate food intake, energy expenditure, and fat storage. Obesity derives from an imbalance between the level of nutrient intake and the amount of energy expenditure. With most individuals, this balance appears to be closely regulated. Molecules known as uncoupling proteins that produce energy

from the cell are implicated in the obesity story because they appear to increase the heat production in the body that results in energy expenditure. Melanocortin signaling appears to be an essential component of normal body weight maintenance. The cloning of the mouse *mahogany* gene, which when mutated darkens coat color, has added to a new appreciation of the melanocortin system. Recent studies now demonstrate that *mahogany* is widely expressed in human tissues and that it also influences energy balance. Expressed in a region of the brain that controls autonomic responses, the *mahogany* mutation can prevent diet-induced obesity in mice. Another recent finding has described central nervous system (CNS) melanocortin in mediating the effects of leptin in the brain to reduce food intake and body weight. Clarifying the CNS mechanisms responsible for the normal regulation of food intake and body weight will lead to discovering how the system may be altered in diseases characterized by energy balance dysregulation, such as obesity. While our genes can create an energy imbalance and affect weight gain by contributing to our susceptibility to overeat and by influencing our propensity to be physically active, environmental and behavioral factors play an important role in determining body weight. Thus, our genetic makeup combined with the environment in which we live present a challenge for us to control our body weight.

Clinical advances in pharmacotherapy, dietary therapy, physical activity and behavioral therapy help to achieve weight loss and reduce risk factors for diseases such as hypertension, high blood cholesterol, type 2 diabetes mellitus, coronary heart disease, and other diseases. For example, it has been demonstrated that weight loss drugs approved by the Food and Drug Administration for long-term use can be helpful aids to dietary therapy and physical activity for some obese patients in enhancing modest weight loss and helping facilitate weight loss maintenance. What is important is that patients are aware of the potential of adverse effects that may occur with the use of drugs and that they continue to be assessed by the physician administering the drug therapy.

Clinical research has advanced weight loss surgery as an option for weight reduction in some patients with clinically severe obesity. Gastric restriction or gastric bypass provides a weight loss intervention for motivated subjects. Behavioral strategies reinforce changes in diet and physical activity in obese adults and have proven effective in producing weight loss in the range of 10% over a period of several months to one year.

To address the major public health challenge posed by overweight and obesity in the U.S., the NIH launched an Obesity Education Initiative, convening an expert panel whose work culminated in the 1998 publication of Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. These guidelines make specific recommendations related to such areas as dietary therapy, physical therapy and weight loss surgery and goals for weight loss maintenance. Advancing knowledge of the molecular basis of obesity continues to offer potential for improved therapeutic approaches to prevent and control this disease.

## Sun and Skin

The sun's rays continue to evoke warnings, especially from medical and public health professionals: wear the appropriate sunscreen and protective clothing, limit exposure time, seek shade. The reason? The sun's ultraviolet (UV) radiation, in the form of UVA (longer wavelength) and UVB (shorter wavelength) rays, has been implicated in skin aging and skin cancers, particularly skin cancers like basal-cell and squamous-cell carcinomas and melanoma. But scientists have also found that UV radiation is not all bad. It stimulates vitamin D production in the skin, and there is some still-controversial evidence of a possible connection to reduced risk of colon and rectal cancer incidence and death from breast cancer.

The challenge for scientists supported by the NIH is to understand as much as they can about both positive and negative effects of UV radiation. What they learn should result in major public health and prevention implications for skin conditions. Several recent NIH-supported studies have focused on the interaction of UV radiation, skin cancer, and vitamin D. These studies lend support to the dominant theory that UV exposure is the main identifiable and preventable cause of skin cancer, and emphasize the importance of UV-induced vitamin D in cell growth and differentiation.

A study supported by NIAMS has looked at the incidence of squamous- and basal-cell carcinoma in patients with the skin disease psoriasis who had participated some 20 years ago in a trial of PUVA therapy, a treatment using UVA and a chemical, psoralen, that sensitizes skin to UV radiation. The scientists found that even in those patients with minimal PUVA or UVB exposure over the past decade, their initial exposure to high-dose PUVA in the trial continued to result in new squamous- and basal-cell cancers. This, say the investigators, shows that PUVA actually began the cancer process rather than simply stimulating a cancer that had already started. A similar study of melanoma incidence in the same population showed that PUVA can initiate this form of skin cancer.

To learn how UV radiation could start and promote skin cancers, researchers examined prostaglandins, cell components that contribute to tissue inflammation. Focusing on the protein COX-2, which helps generate prostaglandins, the study showed that 1) skin-reddening doses of UVB increased levels of COX-2, and 2) skin areas with squamous-cell carcinoma had higher levels of COX-2 than normal, non-sun-exposed skin of the same patients. This UVB-induced increase in COX-2 could be a potential target for drug therapy in preventing skin tumors.

Vitamin D, which is synthesized in the skin as a result of UV exposure, promotes normal growth and differentiation of skin structures, which is somehow disrupted in cancers of the skin. Scientists have found that squamous-cell carcinoma cells failed to differentiate like normal skin cells in response to vitamin D. Their investigations suggest that these cancerous cells lack a substance in their genetic machinery that allows vitamin D to do its developmental work. Despite the success of these studies, the effects of UV radiation still need to be better understood to guide prevention efforts against skin damage. That is why NIH recently cosponsored a major Research Workshop on the Risks and Benefits of Exposure to Ultraviolet Radiation and Tanning. The September 1998 workshop featured experts from government, academia and industry who

reviewed the state of the science on UVA and UVB radiation, and addressed the health effects of various tanning methods and sunscreens. Much more research is needed on the both the harmful and beneficial effects of UV light to improve the basis for public health actions, concluded participants at the Sept. 16-18 Research Workshop on the Risks and Benefits of Exposure to Ultraviolet Radiation and Tanning. A combination of time-of-day adjustments for outdoor activities, structured shade, clothing and other physical blockers, and sunscreens are needed "to allow prudent people to enjoy the benefits of outdoor activities while minimizing the risks."

The workshop, cosponsored by NIH and five other federal agencies, involved basic and clinical researchers, the medical community, and representatives from government, industry and the public. Presentations and discussions covered five major topics: sources and measurement of UV radiation, UV effects on the skin, beneficial effects of UV, ways to produce and enhance the tanning process, and sunburn as an indicator of future biological events. The other workshop sponsors included the Centers for Disease Control and Prevention and the Food and Drug Administration.

## Fetal Alcohol Syndrome

The time that humans spend in the womb is one of the most vulnerable periods in life, in which events that disrupt the fetus's development can set the stage for a future of disability and hardship. Alcohol is among the most potent disruptors of fetal development yet known and is the leading cause of preventable birth defects in the United States. In children exposed to alcohol *in utero*, developmental disruptions may manifest as fetal alcohol syndrome (FAS), disabling deficits that include damage to the nervous system, the command center that includes the brain and regulates everything from movement to memory and learning; retardation; impaired motor coordination; malformations of the face and head; and damage to organs, including the heart. Although the abnormal facial features of FAS sometimes normalize with age, other deficits that are more disabling, such as mental retardation and behavioral problems, persist throughout life.

Designing interventions to deter pregnant women from drinking is among the research efforts underway at the National Institutes of Health (NIH). Equally important are NIH studies that seek to reveal the biological mechanisms underlying FAS, since completely eradicating drinking among pregnant women is a desirable but unrealistic goal. For children subjected to maternal drinking *in utero*, identification of the biological mechanisms underlying FAS may lead to better methods of diagnosing it. More important, understanding these biological mechanisms may help scientists learn how to stop the series of biological interactions that usually would go on to cause FAS once the initial event -- maternal drinking -- has triggered them.

### The Search for the Biological Mechanisms that Underlie FAS

**Trophic Factors.** Trophic factors, naturally occurring substances that promote growth of cells and help them differentiate into specialized body tissues, have long been studied by various research disciplines. FAS researchers are interested in trophic factors because of their role in fetal development. One trophic factor of interest to FAS researchers is vitamin A, since enzymes known to play a key role in metabolizing alcohol also are involved in metabolizing a precursor of vitamin A. FAS researchers are considering the possibility that, when pregnant women drink, the enzyme that normally would metabolize the vitamin A precursor instead metabolizes alcohol. In fact, researchers have found that alcohol reduces levels of vitamin A in pregnant mice.

Some trophic factors affect the growth and survival of nerve cells. Among those interested in neurotrophic factors are alcohol researchers, because the fetal nervous system is especially sensitive to the effects of alcohol, and the development of the fetus's nervous system is among the factors that decides how well that fetus functions after birth. Researchers have done a series of studies on the effects of adding neurotrophic factors to fetal cells exposed to alcohol. In a recent study, researchers simulated the environment of an embryo exposed to maternal drinking in rats' nerve cells. Two neurotrophic factors, brain-derived neurotrophic factor and nerve-growth factor, significantly prevented or reduced loss of nerve cells.

**The Role of the Cell-Death Pathway.** Cell death -- Apoptosis® -- is a normal part of life, as cells die to make way for new ones. Paradoxically, this death is accompanied by growth, and the cell-death pathway -- the series of synchronized molecular interactions that result in *normal* cell

death -- reflects this in its inclusion of mechanisms that promote cell growth and differentiation of cells into specialized tissues. However, various events, such as exposure to toxins, can divert apoptosis from its normal pathway, resulting in abnormal pathways that may spur pathological molecular interactions.

Apoptosis is a very active area of interest in the research community, from aging to cancer. Alcohol researchers are interested in apoptosis because of its implications for growth and development in fetuses exposed to alcohol via maternal drinking. The neural crest, a band of precursor cells present only in the early embryo, develops into nerve cells of the brain and spinal cord and into other organs. Researchers have found that alcohol prematurely initiates apoptosis in this crucial band of cells. Death of neural-crest cells is a normal, necessary part of embryonic development, but scientists have discovered that alcohol-induced apoptosis in the neural crest follows an abnormal pathway likely to contribute to the development of FAS.

The scientists discovered that two steps that usually are present in the normal neural-crest apoptosis pathway are missing in the abnormal alcohol-induced apoptosis pathway. In an animal model, researchers blocked both the alcohol-induced and the normal neural-crest apoptosis pathways by inhibiting a specific enzyme (one of the Acaspase® enzymes involved in cell death in mammals). This finding suggests that, at least at one biological point, the two pathways have in common a specific enzyme. In tracing the steps in the abnormal pathway, scientists are looking for ways of blocking alcohol's effects.

Scientists also have found *in vitro* evidence that oxygen free radicals, including those induced by alcohol -- a known generator of this class of damaging molecules -- lead to excessive neural-crest apoptosis. The scientists strengthened their finding by treating alcohol-exposed neural-crest cells with free-radical scavengers, which significantly improved the cells' viability. These antioxidants appeared to reduce or mitigate the free radicals' harmful effects on the neural crest, a finding that suggests potential for pharmacologic interventions.

### Common Causes

While these findings are immediately relevant to FAS research, they also have implications for studies in other scientific areas -- just as results of studies in other areas have informed the search for mechanisms that underlie FAS. For example, by tracing the abnormal, alcohol-induced apoptosis pathway, FAS researchers also glean information about the normal pathway. Data on apoptosis pathways are important to any research discipline with an interest in the growth and death of cells.

Through continued investigations and information-sharing, FAS researchers are moving closer to understanding the mechanisms that lead to a prevalent, yet completely preventable, disease.

## **Building HIV/AIDS Research Capacity in Uganda**

Tuberculosis, HIV/AIDS, influenza, and dengue fever recognize neither geographic boundaries nor political allegiances, and what happens on the far reaches of the globe can have troubling repercussions for U.S. citizens. Our battle to prevent and cure HIV/AIDS is a dramatic example of the need to mobilize scientific resources to address disease threats and reduce health disparities. AIDS has exacted a profound humanitarian toll, reversed gains in child survival in many African nations, and threatened the economic stability of emerging markets by reducing the number of working age men and women. This situation has an adverse effect on international trade and could potentially affect political stability.

The key to reversing the impact of HIV/AIDS lies in international cooperation and research. Major leaps in our understanding of the biology, epidemiology, clinical treatment and transmission of HIV infection have come from international research. In order to make progress against diseases such as AIDS the United States needs trained and committed research partners in those countries most severely affected by the disease. Recent findings would not have been possible without the contributions of physicians and scientists trained under the Fogarty International Center (FIC) AIDS International Training and Research Program (AITRP). For example, through work supported by this program, scientists at Case Western Reserve University (CWRU) of Cleveland, Ohio, working in Uganda were able to show that a simple, low-cost drug, zidovudine, could dramatically reduce the transmission of the AIDS virus from mother to children. Furthermore, because of its low cost and its effectiveness with a single dose, zidovudine is ideally suited to developing country situations.

Ten years ago, Uganda was in the throes of a devastating epidemic caused by HIV and, after 25 years of civil strife, the health care infrastructure was in a state of collapse. The Ugandan Ministry of Health made HIV/AIDS a priority in rebuilding its public health infrastructure and sought outside assistance in order to counter the HIV epidemic. By providing opportunities for specialized training for dozens of Ugandan physicians over the course of ten years, the AITRP at CWRU played a key role in the Ministry's successful efforts. FIC's program at CWRU was designed to complement and leverage investments by other components of the NIH, namely the National Institute of Allergy and Infectious Diseases, and other federal agencies, such as the Centers for Disease Control and the U.S. Agency for International Development. These agencies were provided expertise and support in areas of research and training not otherwise available.

### A Decade of Progress

After ten years of public education and targeted intervention programs, Uganda is one of the few countries in Africa that can boast of a declining number of people infected with HIV-1. Initially, AITRP objectives were to measure the impact of HIV in Uganda through prevalence and incidence studies and to describe the effect of HIV on other endemic diseases, especially tuberculosis. In the following years, the program's aims evolved to provide additional training in research methodology, medical informatics, and bioethics. Trainees were selected with the help of the Ministry of Health, in order to build up the Ministry's technical expertise in public health, a necessary step in responding to the HIV epidemic. Trainees were able to return to Uganda and

assume key positions within the Ministry. Since the program's beginning in 1988, a total of 11 Master's trainees and 19 Ph.D. trainees began and completed their training at CWRU. Thirty others have benefited from short-term training at CWRU.

Trainees under the CWRU AITRP have participated in a number of important collaborative research activities, including studies of mother-to-child transmission of HIV, neurodevelopment of HIV-infected children, interaction between HIV and tuberculosis, and the number of new HIV infections. Through these studies, researchers have found that active tuberculosis is associated with an accelerated progression of HIV disease. The Uganda-CWRU team also completed a major study of methods to prevent tuberculosis among HIV-infected Ugandan adults. Most recent data reported from Uganda suggests that a single dose of nevirapine given to mother and baby during birth and soon after significantly lowered the rate of HIV transmission at a fraction of the cost of other drugs. This represents a viable and cost-effective regimen for prevention of mother-to-child transmission of AIDS in sub-Saharan Africa. CWRU investigators and trainees are currently preparing for two large studies of HIV vaccine candidates and the role of contraceptives in HIV transmission. The success of these and other projects has depended, and will continue to depend, on the availability and commitment of well-trained Ugandan investigators and technical staff supported by FIC.

Because research and research ethics go hand-in-hand, FIC has welcomed opportunities proposed by grantees to work with communities and to improve their understanding of local considerations in implementing biomedical research. Over the years, CWRU has also established itself as a trusted and important community partner in Uganda. Participants in the AITRP have initiated numerous discussions with medical and public health experts on a range of subjects related to the HIV epidemic. One such set of discussions about current vaccine candidates, potential study designs, and biomedical ethical issues around trials in Uganda led to a public forum in September 1996. This conference sensitized the community and government to the issues underlying the testing of the vaccine. With the current debates about the ethics of AIDS research in developing countries, the CWRU AITRP program has been at the forefront of addressing these issues through their biomedical ethics program.

### Future Steps

Despite the gains of the last decade, the AIDS epidemic is far from controlled. New prevention strategies are needed, including vaccines, effective medications to prevent mother to child vertical transmission, treatment of other sexually transmitted diseases, and enhanced prevention of tuberculosis in people with HIV infection. These needs pose a new generation of challenges for Uganda and other developing countries that will require expertise in fields such as immunology, virology, clinical trials, and behavioral science. In response to these new challenges, AITRP has adapted its goals and redoubled its efforts to develop capacity for new scientific leadership in the field of AIDS prevention. In order to provide a broad scope to its efforts, the AITRP continues to work collaboratively with a number of AIDS-related research programs, not only in Uganda, but also throughout the developing world.

## A Simple Vision Plan

The ability to reach for an object that we see involves a series of neural events that begins with the image on the retina of the eye and ends with signals to the muscles. In the midst of this process, the brain forms a reaching plan. Such a plan could be anchored in the topographic coordinates of the arm, in order to specify the direction and amplitude of the movement. Or the plan could be organized around the coordinates of the eye, because visual information is initially gathered in this form of reference. Understanding how reach plans are represented in the brain could tell us much about the mechanisms and strategies that the brain uses to generate a reach.

The process of visually-guided reaching uses information stored in the visual centers of the brain.

This information is coded in neurons as a three dimensional, topographic map of the image formed on the retina, the sensory tissue lining the back of the eye. Such an organization is a common feature of sensory systems: Sensory inputs such as hearing and touch are also represented as topographic maps in the brain. How do we use this information when we are reaching for an object that we see? How do we transform this sensory information into the motor plan necessary to look towards or reach for an object? How are these seemingly simple plans carried out? Scientists studying this problem in monkeys have identified brain regions, which show that the visual system may play an important role in planning motor actions.

These researchers wanted to know how these plans are encoded in the brain. Using single cell recordings of neuronal activity, they attempted to determine whether reaches are initiated in eye-centered coordinates that indicate where an object is located, or in limb-centered coordinates that would be used to reach for and grasp the object? These experiments with monkeys suggest that the default frame of reference is in a visual-centered map, reflecting the important role the visual system plays in primate behavior.

This work built on earlier studies by other laboratories that examined neurons in the brainstem, which encode and direct eye movements. This work showed that these neurons have a memory for the location of previously-viewed targets. This information was used to direct the eyes to these locations despite changes caused by any intervening eye or head movements. Other experiments indicated that neurons coding the location of sounds also use topographic maps plotted in eye coordinates as the reference frame. In addition, studies in the pre-motor areas of the brain indicated that nerve cells encoding touch also used visual receptive fields as an anchor for the organization of tactile receptive fields.

Past observations by a number of laboratories suggested that the major anatomical pathway for visually-guided reaching begins in the visual cortex and passes through the posterior parietal cortex (PPC) region of the brain. Neurons here play a critical role in coding motor planning for sensory-motor transformations. They receive inputs from a number of sensory modalities as well as signals from motor areas. The scientists studied one region of the PPC called the parietal reaching region (PRR). Neurons in the PRR respond to objects that a monkey can see and reach.

When the scientists began these experiments, they expected the PRR neurons to encode location information in limb-centered coordinates, assuming that for the animal to move the limb toward a target, it needed information on the location of the target with respect to the limb. This was not

the case. The activity of these neurons was driven by inputs that coded information in an eye-centered and not a limb-centered frame of reference.

Why is the brain programmed this way? For one thing it's economical. Even simple tasks require a complex orchestration of eye and hand movements, with the eyes and hands often moving independently to different locations. When you look out at the world, it's a cluttered place. It makes sense for the brain to use visual coordinates to reach for one object in a complex scene. If the brain used limb coordinates instead, it would have to convert the visual coordinates for each object in the scene into limb coordinates. That would require a lot more computation than simply using a single coordinate system to establish a reference frame for all objects in the scene. Another advantage is that visual inputs can be used to modify a reach during its progress. The hand, or limb, is usually visible during reaching and visual cues can be used to make corrections in the reach plan within the same eye-centered coordinate system.

This research has some interesting possible applications. One could use the motor planning information in the PPC to help individuals with paralyzed limbs caused by stroke or trauma. Even though the motor regions in other parts of the brain may be destroyed, the motor planning areas of the PPC may well be intact. One could think about moving the limb, which would encode a motor plan in neurons in the PPC, and this information could be used in conjunction with a prosthetic interface to move the paralyzed limb. Alternatively, the same signals could be used to operate a prosthetic limb or a cursor on a computer screen thereby extending the ability of a paralyzed person to interact with the world. Research is underway with monkeys to determine whether these ideas are feasible.

## **An Appetite for Alcohol**

Alcohol is the most commonly abused of all of the abused substances, resulting in 14 million cases of alcohol disorders in American adults and costing the Nation \$166 billion each year. But is it also a food? Some scientists think so, and in an effort to find the causes of alcohol disorders, they are examining the biological factors that regulate appetite, to see if they apply not only to eating and drinking but also to alcohol consumption. Preliminary studies suggest that they do.

Thinking of alcohol as a food means thinking of it as a source of calories; that is energy. Most of us think of energy as vigor. In fact, that is exactly what energy is: the ability to do work, to move things, like our arms and legs. But what is this elusive phenomenon? We can't see energy; we can only see its effects. To physicists and chemists, energy has an even more complex meaning, although it still has to do with the ability to do work. Although we can't see energy, scientists can measure it. For example, our bodies convert food to energy, which is measured as calories, or the amount of work required to heat the temperature of 1 gram of water by 1 degree.

How the body produces and uses energy from food involves an exquisitely synchronized series of biochemical pathways; that is, predictable sequences of chemical reactions triggered by needs in the cellular environment. For example, if cells need more energy to do their work, pathways that ultimately lead to hunger are triggered. Among these pathways are those that govern appetite and that, like other pathways, respond to signals from their cellular environment. These signals often consist of proteins, including those called peptides,<sup>®</sup> that deliver chemical messages to cells by interacting with them on a molecular level, triggering the next step in the pathway. Genes also are involved in the process, because, although it is the proteins that act as signals, it is the genes that instruct cells on how to produce the proteins in the first place, via DNA codes.

Certainly, alcohol is processed by a host of factors during its journey through the body. But because alcohol is highly caloric, scientists suspect that it may be regulated, at least to a point, by some of the same pathways that regulate the appetitive behavior that governs hunger and other drives. Peptides are integral to these pathways, and research on peptides already known to have a dramatic effect on eating behaviors have led to some intriguing results for alcohol investigators.

### The Tools to Find Out

What had to be in place for scientists to begin studying the relationship between alcohol and the peptides involved in appetitive behavior were animal models. Researchers from different fields have developed strains of animals appropriate for their areas; for example, diabetes investigators developed an obesity mouse model relevant to their work. Alcohol researchers needed animals with a strong preference for alcohol or a strong avoidance of it. About 20 years ago, scientists bred rat strains with these characteristics, and since then have developed others for alcohol research. These animals subsequently have played an integral part in peptide and other studies.

The advent of transgenics, the ability to act directly on specific genes to eliminate or enhance their activity, thereby reducing or increasing the amount of the specific proteins the genes produce, advanced the search considerably. Since the peptides involved in appetitive behavior

are proteins, scientists could now alter the genes that produced peptides, giving a much clearer picture of how specific genes (and their proteins) affect specific behaviors, such as eating. Alcohol researchers, among others, have used transgenic techniques to alter genes involved in producing appetitive peptides, thereby creating animals that serve as important research tools.

One of the most valuable tools for alcohol researchers has been the existing knowledge about peptides and appetite. The study of peptides and appetitive behavior first gained significant momentum in 1982, when researchers studied the effects, on eating, of corticotropin-releasing factor (CRF), a peptide already known to cause the secretion of stress hormones. A major impetus for studies on the role of peptides in appetitive behavior came in 1994, when obesity researchers identified the peptide leptin as a major factor in regulation of obesity. Identification of peptides involved in appetite has progressed at a virtually exponential rate since that time.

#### Where These Tools Have Led

Appetitive peptides are now an active area of study in alcohol research. As the most potent of the known appetite-stimulating peptides, neuropeptide Y (NPY), has received a considerable amount of attention from various disciplines. A recent finding on NPY not only advances alcohol investigators' understanding of its role in alcohol consumption, but also raises important questions for scientists in other fields of appetitive research.

In the recent past, alcohol researchers conducted tests of NPY's effects on alcohol consumption by injecting NPY into animal brains -- a pharmacologic approach. More recently, scientists altered animals' NPY levels transgenically, either eliminating the NPY gene or causing it to produce higher-than-normal levels of NPY. The results were surprising. Since increasing NPY levels greatly increases food consumption in mice, scientists suspected that it would have the same effect on alcohol consumption. In fact, they found that the scenario was completely reversed: transgenic *elimination* of the NPY gene increased alcohol consumption, while enhancing the gene's production of NPY decreased drinking. Furthermore, while transgenic NPY alterations affected alcohol consumption in these mice, it had no effect on their food consumption, unlike pharmacologic alterations (that is, increasing NPY via brain injections).

#### What the New Finding Means: An Exchange of Knowledge Between Fields

Appetitive behavior has critical implications for a variety of diseases and research disciplines. For example, obesity is involved in many serious and costly diseases, such as diabetes and hypertension. More than half of U.S. adults are overweight, and a quarter are clinically obese. The recent findings on NPY raise a number of questions for alcohol researchers and other scientists. Why does pharmacologic, but not transgenic, NPY alteration affect food consumption in mice? Does it mean that the NPY gene is not the key to appetitive food-related behavior, but that the key lies somewhere downstream in the pathway that the gene triggers? Or does it mean that transgenically-induced NPY excess does not reach the levels induced by NPY injections? One of the known effects of NPY is anxiety reduction; do test animals genetically altered to produce too much NPY drink less alcohol because they are less anxious and feel less need for it?

By answering these kinds of questions, scientists will gain clues that can help them trace pathways that end in disease. Doing so increases their chances of one day designing pharmaceuticals that precisely target key points in these pathways to pathology.

## **Helping Couples Conceive**

The desire to have children is a natural and profound basis for many human relationships. For some couples, however, conception proves to be difficult to achieve. Many seek the help of fertility clinics, a remedy that is both expensive and time-consuming.

For those people without serious structural anomalies of the reproductive system, simple lifestyle changes might well solve their problem. Certainly there is an increasing body of scientific evidence to show that events surrounding fertilization and implantation of an egg are enormously sensitive to environmental influences, many of which are within a couple's control. Early work supported by the NIH to develop sensitive fertility assays has led to the development of two important tests: the measurement of human chorionic gonadotrophin (hCG) to detect early pregnancies and of follicle-stimulating hormone (FSH) to measure fertility cycles in women. These assays have been used by NIH-supported scientists to uncover a number of important environmental influences on female fertility. These include the fact that:

1. Cigarette smoking reduces a woman's probability to conceive, but these results are reversed when she stops smoking.
2. Caffeine consumption, such as in colas and coffee, can reduce fertility in women.
3. Exposure to nitrous oxide, the laughing gas<sup>®</sup> used in dentists' offices, can reduce the likelihood of conception for female dental hygienists and dentists, who are exposed to high levels.
4. Douching reduces a woman's chance to conceive.
5. The ideal time for intercourse to lead to conception is earlier than previously thought, reaching an optimal time six days prior to ovulation. In fact, contrary to popular medical dogma, it was found that a woman has little-to-no chance of conceiving by the day after ovulation.

Environmental effects on male fertility have been another topic of exploration. This work has led to the discovery that men taking calcium channel blockers for their hearts have fertility problems because calcium channels are important in sperm activation. Fortunately this effect is reversible when men stop taking the medication.

These findings illustrate that biomedical advances need not always be expensive or involve advanced technology. Simple lifestyle changes can also affect our lives. Defining the environmental components that impede optimal fertility is one way in which the NIH has improved people's lives by providing inexpensive strategies for enhancing their ability to have children.

## Progress in Understanding Alzheimer's Disease

The early Greeks recognized dementia among older persons in 500 BC and thought of it as a normal consequence of aging. In 1906, when Alois Alzheimer studied the brain of a woman, Auguste D., who died at 55 after a 4-year progressive decline into dementia, he reported two striking pathological findings in the brain uncovered by silver staining--neuritic plaques and neurofibrillary tangles. This disease, later named Alzheimer's disease (AD) was thought of as a presenile dementia, since it occurred in a patient younger than 60. In contrast, senile dementia was attributed to decreased blood flow to the brain caused by arteriosclerosis. Research on dementias remained dormant until the 1950s and 1960s, when researchers administered cognitive and behavioral rating scales to elderly subjects and discovered that the majority of the elderly were cognitively normal. Scientists also studied older people with dementia and found that senile dementia was distinct from and more prevalent than vascular dementia, and that the brain pathology of senile dementia closely resembled that of AD. By the 1970s, a unitary concept of AD had been accepted by the medical and scientific community. Recent studies indicate that vascular disease, including strokes, can play a role in the symptoms of some AD patients, but is not the primary cause. These findings laid the groundwork for modern studies of genetic etiology, pathophysiology, and molecular biology of the disease process.

Research on the genetics of AD has provided many new leads in understanding of protein function and the pathways responsible for AD pathogenesis. Over 65 years ago, the initial reports of a familial pattern of inheritance of AD were published. In the intervening years, additional autopsy cases as well as epidemiological studies supported the concept of familial inheritance of AD. But not until 1986, when the gene that codes for amyloid precursor protein (APP), without which there would be no beta-amyloid deposition, was cloned could it be said that the modern era of research into the genetics of AD had begun.

For most individuals the route to AD is a complex one. While for some, perhaps as few as 10% of those with the disease, AD can be attributed to a single gene defect, for the majority of individuals the disease is likely to be multigenic (involve more than one gene). To date, four different genes have been associated definitively with the development of Alzheimer's disease. The first of these genes to be identified was the amyloid precursor protein on chromosome 21. Two other genes for the presenilin proteins--presenilin-1 on chromosome 14 and presenilin2 on chromosome 1--were identified in 1995. Mutations in these genes account for approximately 50% of the inherited early-onset cases. Identifying these genes has led to an explosion of discoveries on the pathways leading to AD. In 1993, a structural variant of the APOE gene on chromosome 19 was associated with late-onset Alzheimer's disease and this variant (or allele), ApoE4, remains the single most significant risk factor, accounting for approximately 50% of the genetic effect in the development of the common, late-onset form of Alzheimer's disease. Recently, it was suggested that the APOE4 allele influences when susceptible individuals will develop Alzheimer's disease; but for individuals surviving to very old age, the genotype had no bearing on whether those subjects developed Alzheimer's disease. Further, it was estimated that regardless of APOE gene status, one-half of those over 100 years of age would not develop Alzheimer's disease. This means that other genetic or environmental factors must be involved.

A number of polymorphisms (genes that exist in more than one variant or allele and where the rare allele can be found in more than 2% of the population) in other genes have recently been advanced as candidate genes for increased risk of late-onset Alzheimer's disease. Among them are genes related to amyloid metabolism, to neurotransmission, to growth factors, to apoptosis and cell death, to the immune system, and to the mitochondrion. So far, none of these associations has been satisfactorily confirmed in other populations.

Much activity is now focused on a small region of chromosome 12 where linkage studies have suggested the existence of an additional major susceptibility locus. A number of candidate genes have been suggested, but the true identity of that gene as yet remains uncertain. The examination of these and other loci will require large samples to detect the modest genetic effects expected. Identifying additional late-onset risk factor genes involved in the development of AD is important because each new gene discovered can point, potentially, toward entirely new mechanisms through which age-dependent neurodegenerative changes occur. In the future, it may be possible to assess a person's risk for developing AD and to identify individuals for early intervention and/or custom-made treatments. Efforts are underway to translate genetic discoveries into an understanding of the molecular pathogenesis of AD and into interventions to slow, prevent, or reverse changes that are responsible for the lesions of AD.

While genetic discoveries were being made, research has been progressing on the pathophysiology and molecular biology of the disease process. Two critical events were the identification of two proteins associated with the hallmark lesions of AD, beta-amyloid in the plaques and tau in the paired helical filament of the neurofibrillary tangles. In the 1980s, beta-amyloid was isolated, purified, and the amino acid sequence determined. This led to discovery of the Amyloid Precursor Protein (APP) gene on chromosome 21 coding for the precursor protein of beta-amyloid. A flurry of experiments followed that continue to this day on the processing, secretion, metabolism, and putative toxicity of beta-amyloid because many scientists believe that accumulation of this protein in brain represents a critical event in the pathogenesis of AD.

The other protein of interest in AD is tau, which normally helps stabilize the structures within the neuron (microtubules) that are important for transport of molecules. When tau is modified by the addition of phosphates, it disengages from the microtubules and aggregates within the neurons as paired helical filaments, the main component of the neurofibrillary tangles. This process disrupts transport of material throughout the cell and may lead to dysfunction or death of the cells. Accumulation of abnormal tau also leads to dysfunction and loss of connections between neurons, a key pathological phenomenon in AD thought to be one of the immediate causes of the dementia. In recent studies, mutations in the tau gene have been discovered in families with other dementias but not in those with AD.

Since the prevalence of dementia increases in an exponential fashion with increasing age and more people are living longer, we can expect to see the numbers of people affected by AD to increase markedly in the next 20 years if we don't intervene to halt the disease process. Failure to intervene will lead to unprecedented demands on families and society, both economic and emotional, to adequately care for the huge numbers of patients predicted to have AD in the 21<sup>st</sup> century. It is important to identify people who are at risk, identify the disease process in its

earliest stages, and aggressively intervene to prevent or impede the progress of this disease. Research is the only solution for finding interventions that could head off a public health disaster.

