NUTRITION, FOOD SAFETY, AND HEALTH

The health of the U.S. citizen depends on the quality and quantity of the country's food supply and the nutrients consumed by individuals. Research is supported which will contribute to the improvement of human nutritional status by increasing our understanding of requirements of nutrients and factors affecting optimal human nutrition. Grants are also awarded in the area of food safety, particularly emphasizing the detection, prevention and control of food-borne disease-causing microorganisms, naturally occurring toxicants and drug residues.

IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH

Panel Manager -Dr. Kenneth G. D. Allen, Colorado State University
Program Director - Dr. Kathleen Ellwood

This program area emphasizes research that contributes to our understanding of appropriate dietary practices throughout the life cycle and factors that affect these requirements such as gender, race and ethnicity. In addition, new insights are needed about factors that affect the attitudes and behavior of consumers toward food. The following areas of research will be emphasized: nutritional requirements including metabolism and utilization for all age groups, bioavailability of dietary components, the interrelationships among dietary components, mechanisms underlying the relationship between diet and optimal health (such as the effect of nutrients on the immune system) and cellular and molecular mechanisms influencing nutritional status (including nutrient effects on gene expression). In addition, this program supports research on identification of obstacles to adopting healthful food habits with particular emphasis on factors affecting consumer attitudes and behavior and development of recommendations for interventions to improve nutritional status. Innovative approaches to these research problems are encouraged.

9800663 Interactive Multimedia CD-Rom for Promotion of Nutrition and Physical Activity in Children
Goran, MI; Reynolds, K.
University of Alabama, Birmington; Departments of Nutrition and Health Behavior; Birmingham, AL 35294
Grant 98-35200-6064; $180,000; 2 Years

In previous USDA funded support the PI has performed extensive studies relating to nutritional requirements in children and the role of diet, physical activity and environment in the etiology of obesity. In this new study we will translate the information that we have gathered from previous supported studies to design and develop IMPACT: Interactive Multimedia for Nutrition and Physical Activity. IMPACT will be a comprehensive, fun, and cultural relevant education tool for children that will be enhanced with appropriate and creative graphics, video, animation and sound. IMPACT will be designed as a potential intervention tool for health promotion and disease prevention and will be designed around a theoretical model designed to change health behavior. Education, self-evaluation and monitoring via direct computer interaction will be important components. The goal is to develop a system in which children are educated and are evoked to make wiser decisions regarding food intake and physical activity behaviors, and ultimately to prevent excessive weight gain and reduce long term nutrition related health risk. The initial 2 years of support for the first phase of this project will be used to design the intervention tool and perform initial testing and evaluation. In future studies we will test the utility of the multi-media tool in a controlled and randomized school-based intervention study. The proposed education and health promotion tool is a novel and creative approach that will be designed to have widespread application for school wide nutrition and general lifestyle education using fun and entertaining instructional activities.

9800675 Do Elderly Women Have a Higher Protein Requirement Than Young Women?
Campbell, W.W.; Evans, W.J.
University of Arkansas for Medical Sciences; Center for Aging; Little Rock, AR 72205
Grant 98-35200-6151; $151,000; 3 Years

The research will assess the dietary protein requirement of young and elderly women and will, for the first time, test the hypothesis that the mean protein requirement of elderly women is greater than that of young women. A double-blinded nitrogen balance study design will be used during three separate 17-day nitrogen balance trials. The subjects will be women aged 18-30 years and women aged 70-85 years. Each of the three 17-day trials will begin with the women consuming a protein-free menu for one day, then defined protein menus providing either 0.50, 0.75 or 1.00 grams protein per kilogram body weight per day for 16 days. Food, urine, and feces samples will be collected during days 11-15 of each trial, analyzed for total nitrogen content, and used to calculate nitrogen balance. At present, insufficient data are available from studies conducted with elderly women to determine a protein requirement with confidence. Yet significant changes in body composition, physical functional capacity, physical activity, food intake, and the frequency of disease in elderly people may all contribute to an altered protein requirement in this population. The results of this research will contribute to our understanding of the requirement for protein, and will be critical to establishing a Recommended Dietary Allowance for protein, of older women. These goals are central to the USDA nutrition, food safety, and health research area and the primary emphasis on nutritional requirements including metabolism and utilization for all age groups.

9800691 Prevalence and Consequences of Vitamin B-12 Deficiency in the Elderly
Allen, L; Haan, M.N.; Green, R.
University of California, Davis; Department of Nutrition; Davis, CA 95616
Grant 98-35200-6100; $190,000; 3 years

Some older people have difficulty in absorbing enough vitamin B-12 from animal products, which are the only natural food source of the vitamin. This research will investigate the prevalence, cause and consequences of vitamin B-12 deficiency in 2000 elderly Latinos

in Sacramento, California. The Latinos are already enrolled in a 5-year longitudinal study of changes in dementia (memory loss), and mental and physical function with aging. There is a general consensus that vitamin B-12 deficiency occurs quite commonly in the elderly but estimates of its prevalence vary from 5-40%. This research will answer the following questions: the prevalence of B-12 deficiency by age in a population-based sample of elderly using traditional (plasma vitamin B-12) and more sensitive (plasma methylmalonic acid and holotranscobalamin II concentrations) indicators of B-12 status; the association between the B-12 status indicators and risk of dementia and loss of mental and physical function, and of high plasma homocysteine levels (an indicator of risk of cardiovascular disease); whether loss of stomach acid is a risk factor for vitamin B-12 deficiency; whether eating more crystalline B-12 and folic acid in fortified foods or supplements protects against B-12 or folate deficiency and their consequences; and whether treatment of individuals with early or late-stage vitamin B-12 deficiency with B-12 supplements improves dementia and cognitive and physical function, and lowers plasma homocysteine. Thus the overall goal is to determine the most useful, early indicator(s) of vitamin B-12 deficiency in the elderly so that the serious, potentially irreversible mental and physical consequences of vitamin B-12 deficiency can be prevented or reversed.

9800690 Assessment of Carotenoid Bioavailability from Plant Sources

Brown, K. H.
University of California, Davis; Department of Nutrition; Davis, CA 95616
Grant 98-35200-6099; $146,000; 2 Years

Green leafy vegetables, such as spinach, are high in beta-carotene, which can be converted in the body to vitamin A. It has been estimated previously that 6 units of beta-carotene are equal to one unit of vitamin A. However, recent studies have introduced uncertainty regarding the appropriate equivalency ratio. Thus, the extent to which beta-carotene from plant foods can provide vitamin A must be reassessed. We will conduct human volunteer studies to measure vitamin A bioavailability from spinach, using a novel stable (non-radioactive) isotope technique to determine the change in vitamin A body stores of volunteer subjects before and after a 60 day period of daily supplementation with either spinach or purified vitamin A in capsules. Both supplements will provide presumably equivalent amounts of vitamin A, (1.5 mg vitamin A equivalents; using the beta-carotene:retinol equivalency ratio of 6:1). The difference in pre- and post-supplementation vitamin A stores will be used to estimate the amount of vitamin A retained in the body from either spinach or the vitamin A capsules. Additionally, a second stable isotope will be administered during the supplementation period to determine whether it is possible to predict the observed change in vitamin A stores in response to spinach or vitamin A capsules after only 5-10 days of supplementation. This would permit a more rapid, simplified assessment of beta-carotene bioavailability for future, related studies.

9800639 FASEB Summer Conference on Micronutrients: Trace Elements

Rucker, R.B.; King, J.C.
University of California, Davis; Department of Nutrition; Davis, CA 95616-8669
Grant 98-35200-6163; $10,000; 1 Year

The FASEB summer conferences are a major vehicle for scientific communication. The conference entitled - Micronutrients: Trace Elements - brings together world leaders who have contributed significantly to our knowledge base regarding the physiological importance of copper, zinc, and selenium metabolism in relationship to their roles as essential dietary constituents. The conference is designed to extend discussions in these areas and share information seminal to future work. The meeting will be held at the Namaste Retreat Center in Wilsonville, Oregon. Specific topics will focus on the cellular metabolism, transport, and functions of copper, zinc or selenium at the molecular levels. There will be two 3 to 3 and 1/2 hour sessions each day (Monday through Thursday). A morning session will be held on Friday. Discussion of new concepts and ideas will be encouraged. A work shop will also be held on recent evidence that has been used to establish the essentiality of boron. The conference is designed to describe and identify new functions and metabolic roles of essential trace elements; a key component in the understanding of human requirements.

9800712 Soy Phytoestrogens and Modulation of Lipoprotein Metabolism

Steinberg, F.
University of California; Department of Nutrition; Davis, CA 95616
New Investigator Award; Grant 98-35200-6437; $144,693; 2 Years

Soy proteins may promote maintenance of optimal health and decrease the risk of chronic heart disease via interactions with the sex steroid hormone system and the cardiovascular system. Soybeans are one of the major agricultural crops in the U.S. and the potential exists for increased demand as soy products are promoted for their nutritional benefits. Phytoestrogens, natural dietary estrogens of plant origin, are components of soy that are postulated to contribute to the plasma cholesterol lowering and anti-atherogenic effects of soy products. However, the mechanisms of action for the protective cardiovascular effects have not yet been elucidated. This project will test hypotheses relating to the estrogenic effects of phytoestrogens on lipid metabolism, to effects of phytoestrogens on the artery wall that affect lipoprotein oxidation and flux, and on expression of the LDL-receptor (LDL-R) gene. The effects of diets containing or devoid of soy phytoestrogens will be tested in atherosclerosis-prone mice. Changes in plasma lipids, arterial cholesterol accumulation and peroxidation, and liver LDL-R gene expression will be evaluated. The mechanism of action of purified soy phytoestrogens on LDL-R

gene transcription will be investigated in cell culture experiments. The results of this project will provide new data that will aid in understanding the mechanism(s) of action of dietary soy phytoestrogens within the hormonal and cardiovascular systems.

9800696 Gender Differences in the Hypolipidemic Effects of Dietary Soluble Fiber

Fernandez, M.L.
University of Connecticut; Department of Nutritional Sciences; Storrs, CT 06269-4017
Grant 98-35200-6052; $155,000; 3 Years

Coronary heart disease is the number one killer in the United States for men and women. Postmenopause in women increases risk for heart disease due to determinental changes in plasma lipids and lipoprotein profiles as a result of estrogen deprivation. Dietary soluble fiber has been shown in numerous clinical studies to decrease plasma cholesterol levels. Apparently postmenopausal women are less responsive to soluble fiber than men. The studies proposed in this investigation are aimed at understanding the secondary mechanisms by which dietary soluble fiber lowers plasma LDL cholesterol and how these responses vary depending on gender. Men, pre- and postmenopausal women will participate in this study and two fiber supplements will be tested. Blood samples will be taken at baseline and at the end of the experiment to measure not only the expected decreases in plasma cholesterol after taking the fiber supplement but to determine certain parameters associated with gender and hormonal status that will be affected. Size and composition of VLDL and LDL, plasma antioxidants, specific plasma proteins involved in the remodeling of lipoproteins and metabolites as markers of cholesterol synthesis and catabolism will be measured. In addition, guinea pig studies will be conducted in parallel to determine the molecular regulation of hepatic enzymes and receptors affected by fiber and gender. The guinea pig is an excellent model that mimics the human situation in terms of plasma lipoprotein distribution and responses to diet. It is postulated that these studies will clarify how gender influences the response to dietary fiber.

9800697 Nutrient Rescue of Aberrant Mitochondrial Metabolism: Vitamin A

Berdanier, C.D.
University of Georgia; Department of Foods and Nutrition; Athens, GA 30602-3622
Grant 98-35200-6049; $188,000; 2 Years

Numerous population studies have identified vitamin A as a nutrient most likely to be deficient in many diets. Some populations appear to be at greater nutritional risk than others due to their particular genetic background. The present project seeks to provide a scientific explanation for this individual variability in vitamin need. It uses a known variant in the mitochondrial genetic material and asks the question of whether this variant dictates a greater than normal vitamin A intake to result in normal tissue function. The results should help us develop nutrient intake recommendations based on genotype such that in the future we will be able to recommend food intakes that potentiate the expression of genes related to good health and a normal productive lifespan while suppressing the expression of those genes associated with degenerative disease and early death.

9800634 Copper, Zinc, Manganese, and Bone Health in Postmenopausal Women

Johnson, M.A.; Lewis, R.D.; Grider, A.
University of Georgia; Department of Foods and Nutrition; Athens, GA 30602
Grant 98-35200-6050; $220,000; 2 Years

Bone loss leads to osteoporosis -- a chronic disorder that increases the risk of bone fractures, particularly in women. It is estimated that 18 to 24 million older women are at risk for poor bone health. Medical costs of osteoporosis are at least $10 billion annually. Drugs such as estrogen can improve bone health, but not all postmenopausal women take estrogen and about 50% discontinue within one year. Preventive strategies involving nutrition may be more economical and have higher rates of acceptance and compliance than certain drugs. Nutritional factors help prevent bone loss by providing bone mineral (e.g., calcium) or by building bone matrix. Copper, zinc, and manganese are three nutrients that build and protect bone matrix. Although it is well known that deficiencies of copper, zinc, and manganese cause severe bone disorders in animals, there has been little research in humans. Many people do not consume enough copper, zinc, and manganese in their diets and thus may be compromising their bone health. Therefore, the objective of this research is to determine whether supplements of copper, zinc, and manganese improve blood indices of bone health in a randomized double-blind placebo-controlled study of postmenopausal women. Blood indices of bone health change rapidly in response to nutrients or drugs and indicate whether bone is being built or lost. This research will identify nutrients that should be studied further in long term studies to determine their role in improving bone health. This research supports USDA's goal of investigating the interrelationships between human nutrition and optimal health.

9800619 Supplemental Zinc During Pregnancy and Fetal Neurobehavioral Development

Caulfield, L.E.
The Johns Hopkins School of Hygiene and Public Health; Center for Human Nutrition; Baltimore, MD 21205
Grant 98-35200-6109; $116,000; 2 Years

Zinc is known to be an essential mineral for adequate growth and development. Previously, we found that supplementing poor Peruvian women with 15 mg zinc/day during pregnancy improved various indices of fetal neurologic development, including patterns of fetal movement and heart rate. These indices of fetal development describe important ways in which the fetus prepares for life after

birth, and can be described using non-invasive electronic fetal monitoring. Despite these findings, the dose of zinc was not sufficient to treat zinc deficiency in these women. In a subsequent study, we are increasing the amount of zinc to 25 mg zinc/day, and are monitoring fetal growth and neurobehavioral development from 20 weeks until delivery in 200 pregnant Peruvian women. In this study, we will measure several indicators of maternal, neonatal and placental zinc status, including red blood cell zinc and metallothionein concentrations, which are advocated as improved methods of assessing zinc status. We will compare different indicators of zinc status to detect change in maternal zinc status brought about by prenatal zinc supplementation. This study will enable us to recommend the appropriate dosage of zinc for prenatal supplements for poor women in the United States, and determine if metallothionein can be used as an indicator for evaluating change in zinc status during pregnancy brought about by prenatal zinc supplementation.

9801023 Optimizing Nutrient Intake for Prolonged Vision

Taylor, A.
USDA Human Nutrition Research Center on Aging at Tufts University; Boston, MA 02111
Grant 98-35200-6057; $188,000; 2 Years

Age-related eye-lens opacification, or cataract, is a major cause of visual disability and blindness. Cataract extraction is the most frequently performed surgery and expenditures for cataract surgery comprise the largest line item in the Medicare budget. Recent studies indicate that dietary or serum antioxidant nutrients (vitamins C and E, and carotenoids) levels are related to risk for opacification. Previously, we conducted a Vitamin C and Cataract study (VCCS) and a “Baseline” Nutrition and Vision Project (NVP). The Baseline NVP was designed to examine relationships between degree of lens opacification and intake of various levels of all antioxidant nutrients and foods. For the Baseline NVP, six hundred and three 54-73 yr old volunteers were recruited. Much of the ophthalmological and dietary data were analyzed. The Baseline NVP data also indicate that risk for opacities is significantly diminished (by 54%) in persons who consume vitamin C supplements for more than 10 years and that daily vitamin C intake of 200-300 mg is associated with this health benefit. We plan to complete analysis and interpretation of the remaining nutritional, ophthalmological and other data we gathered as part of the Baseline NVP. This data will enable us to determine associations between the quantity of intake of specific nutrients, duration of intake of specific supplements and prevalence of major types of opacities. In addition, we will execute the Follow-up NVP. In the follow-up NVP we will reexamine 540 NVP volunteers 5 years after the Baseline NVP examination and determine the extent to which intake of various levels of nutrients, over various periods of time, are associated with delayed onset or diminished progress of major forms of cataracts and changes in visual function.

9800654 Dietary Fat Regulation of Hepatic Glycolytic Gene Expression.

Jump, D.B.
Michigan State University; Department of Physiology; East Lansing, MI 48824
Grant 98-35200-6064; $240,000; 3 Years

Dietary fat is an important macronutrient in the human diet. It provides a source of energy as well as structural components for the synthesis of complex cellular lipids. Dietary fat also regulates cell function through changes in gene expression. Understanding how dietary fat mediates effects on the cell is a central issue in modern nutrition research. Dietary fat regulates hepatic lipid and carbohydrate metabolism through changes in gene expression. While such effects are involved in normal liver function, they may also contribute to the onset and progression of chronic diseases, like insulin resistance and obesity. We do not fully understand how dietary fat regulates liver gene expression. Transcription of the gene encoding the glycolytic enzyme, L-pyruvate kinase (L-PK), is regulated by the type and quantity of dietary fat ingested. Accordingly, L-PK will be used as a model to investigate fat effects on hepatic gene expression. We hypothesize that long chain highly unsaturated fatty acids are metabolized within the liver to generate regulatory molecules that control the activity of factors interacting with the L-PK gene promoter. A molecular approach will be used to define the factors involved in fatty acid control of L-PK gene transcription. A metabolic approach will be used to define the role fatty acid metabolism plays in regulating these factors. These studies will provide a better understanding of how fat in the american diet regulates a key enzyme involved in hepatic carbohydrate metabolism.

9800670 Vitamin A and Hormonal Regulation of Cellular Retinoic Acid Binding Protein I Gene

Wei, Li-Na
University of Minnesota; Department of Pharmacology; Minneapolis, MN 55455
Grant 98-35200-6264; $115,658; 2 Years

Vitamin A, the collective name for retinoids, functions as a group of essential micronutrients for human health. To maintain a homeostatic supply of these micronutrients, well-regulated uptake and metabolism of retinoids must be ensured. A family of cytosolic binding proteins for retinol (CRBPI and CRBPII) and retinoic acid (CRABPI and CRABPII) play important roles in retinoid metabolism. We have created a tissue culture model for the study of how this gene is regulated by vitamin A and thyroid hormones. This proposal is aimed at understanding how the CRABPI gene regulatory machinery responds to hormonal factors such as retinoic acid and thyroid hormones in the established cell culture and animal models. The results from these studies will elucidate how dietary factors may affect the regulatory machinery for CRABPI gene in the context of cell and animal physiology. This information can also be directly applied

to the understanding of how micronutrients such as vitamin A and thyroid hormones may augment human physiological responses that are directly associated with the components of vitamin A metabolic machinery, such as CRABPI.

9801013 Glutathione Peroxidases: Selenium Requirement and Function

Sunde, R.A.
University of Missouri; Nutritional Sciences Group; Columbia, MO 65203
Grant 98-35200-6051; 240,000; 3 Years

Selenium (Se) is an essential trace element that is required by humans and animals. The overall goal of our research is to understand the roles of selenium in health and disease. We have shown that the Se-dependent glutathione peroxidase-1 (GPX) enzyme activity and mRNA levels are effective molecular markers for assessing Se status in humans and animals. We recently extended this molecular approach to more difficult conditions, and showed that se requirements do not increase in pregnant and lactating rats. Our preliminary work suggests that this assessment in blood could be an effective tool to evaluate Se status and set Se requirements. And a GPX1 knockout mouse model now will permit direct testing of these ideas. To better understand Se requirements, and to develop molecular biology techniques to determine human Se requirements, 1) we will use molecular biology approaches to determine Se requirements, with an emphasis on blood. These studies will use rats, but will also extend these methods to human assessment using RT-PCR analysis of blood; 2) we will use the GPX1-knockout mouse model to characterize the role of GPX1 in regulating Se status, and thus to better understand the suitability of using GPX1 expression to set Se requirements. This research will improve our understand of *Selenium* requirements and prevention of Se-responsive diseases. This research will also help to design future sustainable agricultural practices by providing information that will help to reduce release of Se in animal waste, thus protecting the environment.

9800628 Intracellular Copper Trafficking Proteins

Ettinger, M.J.; Aletta, J.M.
SUNY, Buffalo; Department of Biochemistry & Pharmacology; Buffalo, NY 14214
Grant 98-35200-6054; $121,000; 2 Years

It has been known for more than sixty years that copper deficiency impairs brain development, but the mechanisms involved remain largely unknown. We recently found that an enzyme involved in methyl-group metabolism binds copper, and that the levels of this enzyme are significantly decreased in copper deficient mouse brains. The enzyme, S-adenosylhomocysteine hydrolase (SAHH) plays a critical role in all cellular methylation reactions. We have found that inhibiting SAHH markedly inhibits methylations of specific proteins in PC12 cells, a cellular model for nerve cell development. Inhibition of methylation blocks neurite outgrowth from PC12 cells. Copper deficiency also inhibits neurite outgrowth from PC12 cells and methylation of specific proteins from neonatal mouse brain. The hypothesis tested by this project is that copper deficiency decreases SAHH levels and methylation of specific proteins that are required for normal brain development. This research is very significant for U.S. Agriculture because livestock, especially sheep are very susceptible to the effects of copper deficiency on brain influence the central nervous system and the cellular and molecular mechanisms underlying the nutritional requirement for essential trace elements and how copper and related metals influence gene expressions.

9800692 Vitamin E Metabolites in Human Urine: Markers of Bioavailability and Utilization

Parker, R.S.; Swanson, J.E.
Cornell University; Division of Nutritional Sciences; Ithaca, NY 14853
Grant 98-35200-6053; $215,000; 3 Years

Vitamin E is the primary lipid soluble antioxidant in mammalian tissues and is a suspected protective agent against cardiovascular disease and certain cancers. Yet little is known of the factors which regulate the amount of vitamin E in the Body or the forms of vitamin E which the body retains or best utilizes. During the initial grant period we developed sensitive methods of quantifying two types of urinary vitamin E metabolites, one apparently representing unused vitamin E, and the other vitamin E expended as an antioxidant. We discovered that gamma-tocopherol, the most common dietary form of vitamin E in the U.S., is efficiently excreted via the urine. We now propose to (1) compare metabolism of the natural vs. specific synthetic forms of alpha-tocopherol; (2) study the metabolism of an oxidized form of vitamin E considered to result from antioxidant activity; (3) clarify the mechanism and tissue site of vitamin E metabolism; and (4) study metabolism of vitamin E at high doses likely to be used in clinical trials. Our approach includes use of human metabolic studies, forms of vitamin E labeled with deuterium (a stable isotope, which permits discrimination between administered and pre-existing vitamin E), deuterium-labeled standards synthesized during the initial grant period. The proposed research seeks to clarify the metabolic fate of vitamin E in the human, the forms of vitamin E most useful to the body, and consequently more precisely define the human requirement for this important vitamin.

9800686 Dietary Selenium and Immunocompetence in the Elderly

Roy, M.; Kiremidjian-Schumacher, L.; Kim, M.Y.; Gilbride, J.A.
New York University; College of Dentistry; Division of Basic Sciences; New York, NY 10010
Grant 98-35200-6090; $120,000; 2 Years

Aging is associated with a decline in immune function, leading to a higher incidence of cancer and other diseases. This proposal is based on the hypothesis that dietary supplementation with the essential mineral sodium selenite, a dose within the safe and adequate

daily intake recommended by the U.S. Food and Nutrition Board, may abrogate depressed levels or enhance normal levels of immune function in elderly individuals as a result of enhanced expression of functional interleukin-2 receptors (IL2-R). The hypothesis is supported by our previous studies on healthy, young, human volunteers, and young and aged mice. This double blinded, placebo controlled study will focus on the immune responses of free-living Caucasian, African, Asian, and Hispanic Americans of both sexes who are 75 years of age and older, an age group that has a higher incidence of cancer and other diseases. Subjects will take one selenium or placebo tablet/day for 8 weeks. Immune functions will be evaluated before treatment and after 7 and 8 weeks of supplementation. The ability of lymphocytes to respond to stimulation with antigen and mitogen, to differentiate into tumor cytotoxic lymphocytes, and to express IL2-R, which are depressed in aged individuals, will be evaluated. Plasma and lymphocyte levels of selenium and plasma glutathione peroxidase levels (a selenium-containing enzyme) will be monitored. Finally, the ability of the volunteers to develop a delayed hypersensitivity response to a battery of antigens will be evaluated. An enhanced immunocompetence in the elderly through dietary supplementation with selenium may, consequently, result in improved disease-free survival and decreased morbidity.

9800710 A Human Model of Branched Chain Amino Acid Metabolism

Hutson, S.M.
Wake Forest University School of Medicine; Department of Biochemistry; Winston-Salem, NC 27157
Grant 98-35200-6067; $175,000; 3 Years

Human protein metabolism is a dynamic process in which synthesis and breakdown occur simultaneously in a process termed protein turnover. In recent years isotopic tracers have been used to try and answer fundamental questions about how we maintain the constancy of body nitrogen, size and pattern of the free amino acid pool, and set the minimum rate of protein turnover that is essential for life. Another new concept is that amino acids are important as nutrients. Thus there is a need for more accurate estimates of individual amino acid requirements
that include an understanding of the critical "non-protein" functions of the amino acids.

The indispensable branched chain amino acid (BCAA), leucine, is widely used to study human protein turnover. Indeed, several criteria argue for the selection of BCAA as the tools on which to focus for an in-depth understanding of human protein metabolism. We have developed a new model of human BCAA metabolism which takes into account tissue-specific differences in amount and location of key enzymes that control BCAA breakdown. Exciting new results reveal that these enzymes may be located only in very specific cells within tissues. We will use techniques that visualize the proteins in the intact organ to test this hypothesis. This information is expected to have significant impact on the models used for determination of protein and amino acid requirements. To understand the non-protein functions of BCAA, a model system will be set-up to test hypotheses on the involvement of BCAA in body nitrogen metabolism.

9801016 Factors Affecting Eating Patterns & Dietary Change: A Comparison by Ethnicity

Gans, K.M.; Risica, P.M.
Brown University; Center for Primary Care and Prevention; Providence, RI 02912
Strengthening Award; Grant 98-35200-6490; $104,607; 2 Years

There are important discrepancies between national dietary guidelines and the actual diets of Americans. The main goal of this project is to gain insights into factors that affect the eating patterns and dietary change of consumers and determine how these factors differ by ethnicity. The objectives of this proposal are: 1) to compare correlates of low-fat and high-fat eating patterns including ethnicity and other demographic variables, psycho-social variables, health behaviors and specific eating behaviors; 2) to identify predictors of eating pattern change following intervention; and 3) to study the process of eating pattern change - identifying which eating behaviors were more likely to be changed and maintained over time, and whether this deferred by ethnicity, gender, socioeconomic status and other demographic characteristics. The data to be used in this proposed project were collected during the evaluation of two randomized intervention trials in New England. The data sets include cross-sectional and longitudinal data assessed at three time points on over 10,000 individuals from a variety of demographic subgroups. Each of the study ethnicity specifically for Hispanics and Non-Hispanic Whites. This project will yield data that allow for identification of population subgroups who would benefit from nutrition education programs and determine which factors might motivate different people to make dietary changes. It will also provide information on the specific food sources and eating behaviors present in these subgroups, which allows for targeting of education effects.

9800699 Mechanisms of Leptin Resistance in Diet-Induced Obesity

Gettys, T.W.
Medical University of South Carolina; Department of Medicine; Charleston, SC 29425
Grant 98-35200-6089; $170,000; 3 Years

Accumulation of excess adipose tissue is a known risk factor for hypertension, heart disease and noninsulin-dependent diabetes mellitus. The epidemic of obesity in Western society is strongly associated with consumption of high fat diets. A subset of this population is sensitive to the diabetes promoting effects of high fat in that their obesity progresses to insulin resistance and eventually diabetes. A similar variation in sensitivity to high fat diets has been documented among mouse strains. For instance, C57BL6/J mice develop an obese/diabetic syndrome when reared on high fat diets, while mice of the A/J strain develop none of these pathologies. Thus, the C57BL6/J mouse provides an excellent model to study the developmental stages of an obese/diabetic syndrome that is highly analogous to human noninsulin-dependent diabetes mellitus. The implication of these studies is that high fat diets inhibit the actions

of the recently identified hormone, leptin, to limit food intake and/or stimulate energy utilization in the fat-sensitive C57BL6/J strain. Thus, we will test the hypothesis that high fat diets limit the ability of leptin to regulate specific genes that are necessary to respond to increased caloric density and avoid obesity. Identification of such genes and understanding how they become dysregulated in fat-sensitive mouse strains will facilitate identification of their human homologues, and provide a way to screen and intervene in individuals predisposed to develop diabetes when consuming a typical high fat western diet.

9800658 Nutritional Regulation of the Human Fatty Acid Synthase Gene

Moustaid, N.
University of Tennessee; Department of Nutritional and Agricultural Experiment Station; Knoxville, TN 37996
Grant 98-35200-6039; $180,000; 3 Years

Because several diseases have been linked to high fat intake, dietary recommendations include reduced fat consumption and a modest increase in dietary carbohydrates. However, a larger proportion of our population is becoming obese, suggesting a possible role of increased carbohydrates, in addition to fat intake, in the promotion of obesity. Accordingly, the proposed research is designed to improve our understanding of optimal nutrition by elucidating mechanisms by which specific nutrients-in particular carbohydrates and fats-influence fat-metabolizing genes in human fat cells. To address these issues, we used as a marker for fat synthesis, the fatty acid synthase gene, a key gene in the production of fatty acids from carbohydrates. Viable and metabolically active fat cells were obtained from patients undergoing elective or abdominal surgery. This research uses modern cellular and molecular biological techniques to understand how different types and amounts of carbohydrates and fats regulate the fatty acid synthase gene, and how the amount of carbohydrates converted into fat by human fat cells is controlled by these nutrients. Identification of factors involved in nutritional regulation of fat-metabolizing genes may provide insight into novel therapeutic approaches for the treatment of obesity and other metabolic disorders. In conclusion, our findings using this human fat cell system, together with clinical trials with these nutrients should be considered in optimizing the dietary recommendations to improve human nutrition for optimal health, as emphasized by the USDA.

9801032 The Role of Cu-ATPases in Copper Absorption and Excretion

Harris, E.D.
Texas A&M University; Department of Biochemistry and Biophysics; College Station, TX 77843-2128
Grant 98-35200-6055; $250,000; 2 Years

We have been studying the role of copper in human nutrition. At present very little is known about the biochemistry of copper although the need for this mineral to maintain the health of practically all forms of life is well established. While the incidences of copper deficiency are very low in the U.S., there is still an urgent need to learn more about the amount of copper we take in; specifically, how much copper is safe and adequate, and how does our system regulate its need and guard against toxicity. Towards these goals we have been studying the mechanism for absorbing and distributing copper to essential organs and tissues. We have focused on the molecular events that transpire when copper passes through the gut, moves into the blood stream, and eventually reaches its target organs. These events combined with excretion keep an individual in copper balance. There is a specific enzyme protein (Cu-ATPase) that transports copper across membrane surfaces such as the gut and brain, and is instrumental in maintaining copper homeostasis at the cellular and systems levels. We want to learn more about this protein, specifically its role in copper absorption and excretion, and its location in cells. An exciting finding is that there may be multiple forms of the Cu-ATPase that are regulated by copper. These studies will lead to a better understanding of the essential role of copper in human nutrition and perhaps alert us to dangers when dietary copper is in short supply.

9800671 Regulation of Lactase Activity by Trophic Feeding in Preterm Infants

Shulman, R.J.
Department of Pediatrics; Baylor College of Medicine; Houston, TX 77030-2600
Grant 98-35200-6056; $141,000; 3 Years

Gastrointestinal function in the preterm infant is underdeveloped compared with that in the term infant. There is a great need to develop new strategies that will enhance gastrointestinal maturation in the preterm infant in order to reduce the severity of illnesses and high cost associated with caring for these infants. To accomplish this goal, direct measurements from studies in the preterm infant are needed to understand the factors associated with gastrointestinal development. For example, lactose provides approximately 40% of the total energy in human milk and also can either enhance or hinder the absorption of other nutrients. Lactase is the enzyme responsible for digesting lactose. Despite the importance of lactose in the diet of the preterm infant, we do not know yet what accounts for the normal developmental rise in lactase activity. The goal of this study is to identify the mechanism(s) that account for the increase in lactase activity caused by feeding the preterm infant. To accomplish this goal, preterm infants will undergo measurement of lactase activity and lactase mRNA levels using cells obtained from the intestine. These cells will be analyzed in order to ascertain if the developmental rise in lactase activity is mediated through increases in lactase mRNA. By using new and novel direct techniques, these investigations will provide insight into the biology of gastrointestinal development in the preterm infant. The results of these studies will aid in the feeding of preterm infants.

9800667 24,25-Dihydroxyvitamin D_3 and Intestinal Mineral Transport

Nemere, I.

Utah State University; Department of Nutrition and Food Sciences; Logan, UT 84322-8700
Strengthening Award; Grant 98-35200-6466; $83,147; 3 Years

The overall project goal is to further our understanding of how vitamin D metabolites affect calcium and phosphate transport in the intestine, thereby influencing nutritional status. The most studied metabolite, 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$, appears to block the rapid effect of the stimulatory metabolite, suggesting both have an important function in maintaining appropriate mineral balance. Using perfused intestinal segments (duodena), physiological levels of 24,25$(OH)_2D_3$ will be tested for effectiveness in inhibiting 1,25$(OH)_2D_3$-stimulated mineral transport. These data may be useful in correcting conditions of excessive blood levels of calcium (hypercalcemia) or phosphate (hyperphosphatemia). The project will then begin to undertake an investigation of the mechanisms of action of 24,25$(OH)_2D_3$ by determining which intestinal cell compartment contains a specific binding protein (receptor) for the metabolite, the specificity of the receptor for various metabolites, and other parameters of binding. Finally, the study seeks to purify the receptor as a means to promote future studies at the levels of the gene in both normal human populations, as well as those with inheritable disorders such as hypophosphatemic rickets. These objectives will address the cellular and molecular mechanisms that influence nutritional status.

ENSURING FOOD SAFETY

Panel Manager -Dr. James Lindsay, University of Florida
Program Director - Dr. Kathleen Ellwood

Safety of food products is of paramount importance to the producer, processor, distributor, and consumer. The research program in food safety focuses on research questions involving disease-causing microorganisms, their products, naturally occurring toxicants or drug residues which contaminate food. The program emphasizes detection, prevention and control. Projects may focus either on pre- or post-harvest/slaughter origin of the microbial agent or residue.

9802737 Rapid Detection of Brevetoxin and Ciguatoxin Using Recombinant Na+ Channels

Critz, S. D.; Hartmann, H.
University of South Alabama College of Medicine; Department of Structural and Cellular Biology; Mobile, AL 36688
Grant 98-35201-6208; $90,000; 2 Years

Contamination of shellfish by a marine toxin known as brevetoxin periodically threatens the health and safety of seafood consumers. Brevetoxin poisoning typically causes neurological and gastrointestinal disturbances that last 3 to 4 days but fatalities have been reported in severe cases. There are not any known antedotes for poisoning and consequently toxin levels must be rigorously monitored to prevent ingestion of tainted seafoods. This proposal will develop rapid and sensitive methods to test for brevetoxin and ensure seafood product safety.

9802745 Role of Putative Pathogenicity Island in *Campylobacter jejuni* Virulence

Joens; L. A.
University of Arizona; Department of Veterinary Science; Tucson, AZ 85721
Grant 98-35201-6195; $190,000; 3 Years

Incidence of campylobacteriosis in man has risen dramatically in the past 10 years and passed salmonellosis as the number one disease acquired by consuming contaminated food products. It is estimated that *Campylobacter jejuni* causes 3 million cases per year; with a cost due to treatment and loss of productivity greater than 1 billion dollars. Clearly; understanding mechanisms by which this major pathogen causes disease is invaluable. Nevertheless; few factors associated with *C. jejuni* virulence have been identified. Progress in this important research area has been hampered by the lack of genetic tools to examine or identify virulence factors. To date; roughly ninety *C. jejuni* genes have been characterized; only six of which are related to virulence. Recently we have isolated an iron uptake system encoded within a putative pathogenicity island from virulent *C. jejuni*. Genes within this island may be influential in defining the pathogenicity of the agent that will lead to control measures for decreasing the incidence of campylobacteriosis.

The long range goal of the proposed research is to evaluate genes contained within a putative pathogenicity island in *C. jejuni* for their role in virulence. Sequence analysis reveals an area that for encodes two disparate physiologic function; an iron uptake system; and a cell wall biosynthetic pathway; suggesting that maintenance of this unique island may not be due to environmental iron limitations alone. The island may encode other functions and comprise not only an iron uptake island but a pathogenicity island. Consistent with these observations we hypothesize that this locus comprises a pathogenicity island which contributes to *C. jejuni* virulence.

9802739 Strategies to Eliminate and Prevent Microbial Contamination of Food Products

Compadre, C. M.; Breen, P. J.; Zhou, X.
University of Arkansas for Medical Science; College of Pharmacy; Food Safety Group;
Little Rock, AR 72205
Grant 98-35201-6438; $70,000; 1 Year

While the American food supply is considered among the safest in the world; recent estimates suggest that as many as 9,000 deaths and 6.5 to 33 million illnesses in the United States each year are food-related; with medical costs and productivity losses ranging between $1.8 billion and $4.8 billion annually. The need to develop strategies to eliminate and prevent microbial contamination of food products is highlighted by factors such as: emerging foodborne pathogens and pathogenic strains; new and more frequent outbreaks of foodborne disease; and the increased susceptibility to foodborne infections of population groups with lowered immunity. Previous research in our laboratory indicated that cetylpyridinium chloride (CPC); a chemical safely used for over 30 years in oral hygiene products; was able to reduce *Salmonella* and other bacterial contamination from poultry tissues. CPC was also effective in preventing bacterial attachment; and thus has potential to reduce the risk of cross-contamination. The ultimate goal of the research in this proposal is to develop effective methods to control; eliminate and prevent microbial contamination of food products. The specific aims of this project are: 1) To determine the effectiveness of CPC for decontamination of fresh fruits and vegetables; and 2) To assess the organ.

9802830 Bacteria for Competitive Exclusion of *Salmonella enteric* Species in Chickens

Joerger; R. D.
University of Delaware; Department of Animal and Food Sciences; Newark, DE 19717
New Investigator Award; Grant 98-35201-6265; $185,000; 3 Years

The community of bacteria inhabiting the intestinal tract can prevent or impede the establishment of undesirable bacteria such as *Salmonella enterica* subspecies. Newly hatched chicks lack an intestinal bacterial community. Exposure to intestinal bacteria from adult chickens potentially heightens the resistance of young birds to infection with *Salmonella*. Such bacterial preparations can therefore contribute to increased microbial food safety of poultry products by reducing the number of chickens in a flock that carry *Salmonella* into the processing plant. The optimal composition of an effective bacterial preparation is currently not known. The goal of the proposed research is to identify and isolate bacteria from the intestinal tract of chickens and to eventually test their effectiveness in reducing colonization of young chicks with *Salmonella*. The research will focus on the bacteria that are associated with the inner surface of one of the chickens intestinal organs, the cecum. Since a considerable fraction of intestinal bacteria has been refractory to isolation in pure culture, the bacteria will initially be identified without culturing, using genetic means. Identification will be achieved by sequencing of 16S rRNA genes amplified from total DNA extracted from the cecal bacterial community. Denaturing gradient gel electrophoresis of amplified DNA and in-situ hybridization with specific probes will provide information on the diversity, location and prevalence of bacteria within the ceca. Once the identity of the cecal bacteria is known, culture conditions for their isolation can be designed and their value in prevention of *Salmonella* carriage in chickens can be assessed.

9802839 Fluorescence-Based Chemical Sensor for Saxitoxin

Gawley, R. E.; Leblanc, R. M.
University of Miami; Department of Chemistry; Coral Gables, FL 33124-0431
Grant 98-35201-6209; $95,000; 2 Years

Saxitoxin is the primary constituent of the so-called paralytic shellfish poisons (PSPs). Currently, contamination of shellfish beds by PSPs is monitored by mouse bioassay. Recently, we have discovered a molecular receptor that “signals” the presence of saxitoxin in solution by emitting light (technically known as enhanced fluorescence emission). This type of phenomenon is of considerable interest to the chemical community in general, but regarding saxitoxin, it could be the key to developing a photochemical sensor that would complement mouse bioassay. The first stage of this development, funded by this project, is to study the details of the “recognition” and emission phenomenon between saxitoxin and its receptor, both in solution and at a surface (the air-water interface). We will begin by examining the fluorescence emissions quantitatively, then modifying the receptor to optimize its binding and signaling properties. We will modify the receptor to make it amenable to incorporation into a monolayer that, when spread over the surface of water, can be studied in a similar fashion. Studying the phenomenon in a monolayer is a prerequisite to development of a sensor using fiber optics.

9802757 Defining Genomic Sequences Specific to Virulent *Vibrio vulnificus* Strains to Assess Risk

Tamplin, M.; Gulig, P. A.; Parveen, S.
University of Florida; Department of Family; Youth and Community Sciences; Gainesville, FL 32611
Grant 98-35201-3309; $90,000; 1 Year

Vibrio vulnificus is the leading cause of reported human death in the US caused by the consumption of sea foods. Since its discovery, *V. vulnificus* has had a significant impact on public health policy, food regulations, and industry practices. Currently, there is no practical test to determine if seafood products contain hazardous strains of *V. vulnificus*. We propose to solve this problem by defining DNA sequences specific to virulent strains, and then developing simple DNA probe test(s) that can be used by industry and public health organizations to assess risk. We will use two techniques to identify segments of DNA that are unique to virulent strains, 1) by “subtracting” DNA of non-virulent strains from virulent strains, thereby identifying virulent-specific DNA sequences, and 2) by allowing the mouse model to directly select for strains that have acquired DNA sequences from virulent *V. vulnificus* strains that are randomly cloned into non-virulent strains. These approaches will produce virulence-specific gene probe(s) that can be widely used to assess *V. vulnificus* hazards in seafood products. We anticipate that this information will be integrated in ongoing CDC-FDA-State efforts to determine the epidemiology of *V. vulnificus* infections and to develop effective interventions to reduce risk of *V. vulnificus* disease.

9802817 Regulation of Lipopolysaccharide Micro Heterogeneity

Guard-Peter, J.
USDA Agricultural Research Service; Southeast Poultry Research Laboratory; Athens, GA 30605
Grant 98-35201-6281; $125,000; 2 Years

Bacteria that contaminate food are genetically capable of altering their growth properties and cell surface properties in order to infect susceptible people or animals. One food-Borne bacterium that is especially capable of contaminating the contents of eggs is *Salmonella enterica* serovar Enteritidis (SE). It has been shown that certain strains of SE enter an accelerated phase of growth when environmental conditions provide appropriate signals to the bacterial cell. During enhanced growth, many of the cell surface features of SE alter drastically, which contribute collectively to an enhanced ability of the bacteria to grow to high numbers in organs, as measured by increased recovery from the organs of chicks, mice, and eggs. The most prominent molecule composing the outer membrane of SE is lipopolysaccharide (LPS). Previous research indicated that changes in LPS structure can be used to detect strains that are more capable

of attaining full virulence. Current research to be conducted under this grant is directed towards understanding the genetic changes that result in strain variation, as measured by the ability of strains to generate particular LPS structures while maintaining accelerated growth. This work is important because it will lead to a better understanding of environmental conditions that favor outgrowth of new strains of SE. Since certain LPS structures alter the production of proteins on the cell surface, this research should also contribute to a more complete understanding of the immunological properties of virulent SE and to the development of improved vaccines.

9802750 Mechanism for Inactivation of Microorganisms by High Oxidation Potential Water

Hung, U.-C.; Brackett, R. E.; Erickson, M. C.
University of Georgia; Department of Food and Technology; Griffin, GA 30223-1797
Grant 98-35201-6196; $120,000; 2 Years

Reported cases of outbreak of food-related illnesses as well as severity of infection and cost of treatment are on the increase. An estimated 6.5 to 33 million people are infested annually in the U.S., out of which 9,000 die. The high oxidation potential (HOP) water has been reported by scientists in Japan to have strong bactericidal effect on most pathogenic bacteria. A major advantage of HOP water is that no chemical except water (with very dilute NaC1) is used. Therefore it has less adverse impact on the environment. Also, the treated food is not exposed to heat treatment and will experience minimal change in quality.

The overall objective of this project is to study the fundamental principles involved in the inactivation of food microorganisms with HOP water. HOP water with different properties will be used to treat five strains each of three pathogenic bacteria (*Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli* O157:H7). The HOP water with the most effective combination of properties will then be used to evaluate the effect of different organic materials in food systems on its antimicrobial effect. Bacteria inactivation on food surfaces with HOP water will be evaluated using inoculated food samples. The application of this technology will ensure food safety at reduced cost, high food quality and reduced danger from food borne illness.

9802819 Screening Corn for Resistance to *Aspergillus flavus* and Aflatoxin Accumulation

Russin, J. S.; Stetina, S. R.
Southern Illinois University; Department of Plant & Soil Science; Carbondale, IL 62901
Grant 98-35201- ; $100,000; 2 Years

Two traits in corn genotypes will be used to identify potential resistance sources: sporulation by *Aspergillus flavus* on intact or endosperm-wounded kernels, and accumulation of norsolorinic acid (NOR) in kernels inoculated with *A. parasiticus* isolate SK1. NOR is an orange-pigmented intermediate in the aflatoxin biosynthesis pathway. Genotypes that exhibit minimal sporulation and accumulation of orange pigment will be tested for aflatoxin resistance in controlled laboratory inoculations. Genotypes from this pool that prove to be resistant will be evaluated for resistance in field trials. Those that show resistance will be examined for mechanism of resistance. Surface wax will be removed from intact kernels of these resistant genotypes, as well as standard susceptibles, and weighed. Resistant genotypes with large amounts of wax will demonstrate that wax acts as a physical barrier to infection by *A. flavus*. Wax removed from kernels will be bioassayed for antifungal activity against *A. flavus*. Resistant genotypes with antifungal properties will demonstrate that wax acts as a physiological barrier to infection by *A. flavus*. Protein profiles determine whether proteins unique to, or in greater concentration in, resistant genotypes are involved in resistance to *A. flavus*.

9802756 *Listeria monocytogenes:* Ozone Inactivation

Martin, S. E.
University of Illinois; Department of Food Science and Human Nutrition; Urbana, IL 61801
Grant 98-35201-6217; $95,000; 2 Years

The objectives of this study are to examine the effects of ozone (O_3) on the pathogen *Listeria monocytogenes*. Ozone is one of the most powerful oxidizing agents (52% stronger than chlorine) and is effective against a broad spectrum of microorganisms, including viruses, bacteria, yeast and molds. Ozone has recently been recommended for approval as Generally Recognized As Safe (GRAS) in food production. Little or no research has been performed on the influence that ozone has on the destruction of *L. monocytogenes*. This proposal will determine important parameters of ozone-induced injury and death of *L. monocytogenes*. The optimal conditions of ozone exposure for listerial death will be determined. Initial variables will include time, temperature and ozone concentration. Injury and death will be determined using a dual plating procedure, and by the release of subcellular components. The thermodynamics of ozone inactivation will be determined. Any differences in the phase of growth on the susceptibility of *L. monocytogenes* to ozone will be examined. Recovery of ozone-injured listerial cells will be studied, as will be the site(s) of ozone damage. The influence of ozone in reducing listerial counts in a food product (cabbage) will also be determined. The influence of ozone on the enzymes catalase and superoxide dismutase in *L. monocytogenes* will be examined immediately following exposure and during recovery.

9802811 Persistence of *Salmonella typhimurium* in Swine

Isaacson, R. E.
University of Illinois; Department of Veterinary Pathobiology; Urbana, IL 61802
Grant 95-37201-1991; $240,000; 3 Years

S. typhimurium is one of the major causes of salmonellosis in humans. Pigs persistently infected with *S. typhimurium* are one of the major reservoir of this pathogen. Generally, pigs persistently infected with S. typhimurium are asymptomatic. One means to reduce the risk of food borne infections caused by *S. typhimurium* is to prevent pigs from becoming persistently infected. This project is designed to understand the mechanisms promoting persistent infections. The initiation of infection requires the attachment of *S. typhimurium* to the lining of the small intestine. There are two types of cells int he small intestine that are targets for attachment: epithelial cells call enterocytes and epithelial cells call M-cells. Our current hypothesis, which is based on our previous experiments using mutant *S. typhimurium* that do not attach to enterocytes, is that attachment to enterocytes is important for the development of persistent infections while attachment to M-cells results in disease. The goals of this study are to confirm that our non-adhesive mutants indeed do not attach to enterocytes in pigs but retain the ability to attach to M-cells. Furthermore, we have found that a novel mechanism allows *S. typhimurium* to sense its location in the intestine and turn on a set of genes that promote its ability to colonize enterocytes and this results in persistent infections. We plan to create additional mutants that no longer can control these genes and determine whether the mutants have lost the ability to cause persistent infections.

9802730 Analysis of the Osmotic Regulation of Thermotolerance in *Salmonella* and *E. coli* O157:H7

Csonka, L.
Purdue University; Department of Biological Sciences; West Lafayette, IN 47907-1243
Grant 98-35201-6219; $185,000; 3 Years

The addition of moderate or high concentrations of salts or sugars can enhance the high temperature tolerance of food pathogenic bacteria such as *Salmonella* and *E. coli* O157:H7. This enhanced thermotolerance is manifested both as increased growth rate at non-lethal high temperatures and increased survival at otherwise lethal high temperatures. These observations indicate that the addition of salts or sugars to food products as preservatives or flavor components can compromise the efficacy of high temperature treatment for the inactivation of contaminant organisms. We found that betaine, which is found at high levels in edible plants such as spinach and cereal grains, block the ability of salt additives to increase the thermotolerance of bacteria. Part of the research project will be to carry out a comprehensive characterization of all available structural relatives of betaine for their ability to counteract the induction of increased thermotolerance by salt additives in food pathogenic bacteria. This procedure may uncover new food additives that might be used to increase the efficacy of thermal inactivation in food contaminating bacteria. A second component of the project will be to discover genes which are involved in the induction of increased heat tolerance by salt. The understanding of the mechanistic connection between high salinity and increased thermotolerance has the potential application that it might lead to the design of new antimicrobial compound that could lead to decreased survival of food pathogens during thermal processing.

9802814 Molecular Biology of Aflatoxin Biosynthesis in *Aspergillus flavus*

Woloshuk, C. P.
Purdue University; Department of Plant Pathology; West Lafayette, IN 47907
Grant 98-35201-6218; $160,000; 2 Years

Aflatoxins, Produced by the fungus *Aspergillus flavus*, are toxic and carcinogenic compounds contaminating a variety of food products. In addition to the significant health risks associated with aflatoxins, there is also an economic burden. Millions of dollars are spent each year to test potentially affected food, including corn, peanuts, figs, tree nuts and milk. Aflatoxins are products of fungal secondary metabolism. The genes involved in the biosynthesis of aflatoxin are grouped together in a single cluster. The cluster contains at least 18 genes that code for the pathway enzymes and for regulation of aflatoxin biosynthesis. This research project will investigate an unusual mutation in *A. flavus*. Strain 649 has a DNA deletion at the afl-1 locus that includes the entire cluster of aflatoxin biosynthesis genes. Diploids formed by crosses between strain 649 and aflatoxin-producing strains do not produce aflatoxin. The goal of the research is to determine the mechanism responsible for this suppression of aflatoxin biosynthesis. The specific objectives are to isolate and characterize the DNA at the deletion break-junction region in strain 649, and to determine the involvement of the regulatory gene *aflR* in repression of aflatoxin biosynthesis. This research will impact agriculture by furthering our understanding about the regulation of aflatoxin biosynthesis and contribute information leading to development of new strategies for eliminating aflatoxin contamination.

9802797 Modeling Food Fluctuating Microbial Populations and Their Aperiodic Outbursts

Peleg, M.; Horowitz, J.
University of Massachusetts; Department of Food Sciences; Amherst, MA 01003
Grant 98-35201-6271; $90,000; 2 Years

The total number of microorganisms, or of specific types, which are encountered in raw beef or dairy products for example tend to fluctuate. Usually such daily or hourly fluctuations are within a specified range and hence raise little or no safety concern. Only occasionally, and in some cases without an apparent cause the numbers encountered are unusually high and may be considered a safety

problem. We plan to analyze the fluctuations pattern, and by mathematical models and statistical methods, to estimate the probability of the occurrence of such an outburst. In other words, we propose to convert the apparently random sequence of counts into a set of probabilities of encountering outbreaks of a magnitude of safety concern. These calculated probabilities can then be used as indication of an impending microbial outbreak, and as a tool to assess quantitatively the efficacy of preventive methods in reducing the risk.

9802726 Genomic Analysis of *Escherichia coli* O157:H7 Populations from Cattle and Humans

Benson, A. K.; Hutkins, R. W.
University of Nebraska; Department of Food Science and Technology; Lincoln, NE 68583-0919
Grant 98-35201-6215; $150,000; 2 Years

Genetic fingerprinting of *E. coli* O157:H7 strains from cattle herds indicates that certain strains can be repeatedly isolated from a given herd over time (persistent population) despite the fact that other genetically distinct *E. coli* O157:H7 strains may be introduced into the herd but are unable to displace the persistent population. These results suggest that persistent strains are better able to survive and/or propagate in these environments. Are there particular herd management practices that have selected for these persistent populations and are these strains genetically distinct from those isolated from infected humans? We have developed a powerful technique, termed high-resolution genotyping (HRGT), that permits identification of even minor genetic differences between different *E. coli* O157:H7 strains. This procedure will be used to develop a database for rigorous assessment of the genetic relatedness of persistent and non-persistent and non-persistent isolates will be examined in further detail (full genome coverage) by HRGT to identify genetic differences that may be related to persistence. The genes that are marked by these differences will then be examined to begin assessing how their function may contribute to persistence and whether particular herd management practices may have played a role in selecting for alterations in these genes. Ultimately these results may provide a rational basis for understanding the impact of herd management practices on the population structure of this organism.

9802804 Antimicrobial Use and Emerging Resistence of *Salmonella typhimurium* in Dairy Cattle

Warnick, L. D.
Cornell University College of Veterinary Medicine; Department of Clinical Sciences; Ithaca, NY 14853
New Investigator Award; Grant 98-35201-6211, $120,000; 2 Years

Antibiotic-resistant *Salmonella* infections in human are an increasing public health problem. The use of antibiotics in food producing animals for disease prevention or treatment and to enhance growth potentially selects for resistant *Salmonella* strains which may be transmitted to humans. Livestock investigations are needed to identify practices associated with the emergence of *Salmonella* resistant to antibiotics currently important in human medicine.

This project will investigate the effect of antibiotic treatment of clinical salmonellosis in dairy cattle on the occurrence of antibiotic-resistant *Salmonella typhimurium*, a major animal and human pathogen. This will be done by identifying dairy herds with *Salmonella typhimurium*-infected cattle based on diagnostic laboratory culture results, obtaining antibiotic treatment information from farm records and collecting fecal samples from cattle on the farm for *Salmonella* isolation and determination of resistance patterns. The association between cattle from the same herd will be analyzed. The characteristics of study farms will be typical of large segment of the U.S. dairy industry.

The study results will provide valuable information on the emergence of antibiotic-resistant *Salmonella typhimurium* and specific drug-use practices which are associated with resistance. This will allow implementation of changes on farms designed to reduce the development of antibiotic-resistant bacteria and thereby decrease foodborne or direct transmission of resistant strains from dairy cattle to people. The project will also contribute data needed for policy decisions by regulatory agencies related to antibiotic use in food producing animals.

9802753 Identification of Human Enteric Viruses in Foods and Fooborne Disease Outbreaks

Jaykus, L.; Moe, C. L.
North Carolina State University; Food Science Department; Raleigh, NC 27695-7624
Grant 98-35201-6216; $140, 000; 2 Years

Enteric viruses are significant human pathogens, recently ranked fifth and sixth amongst identified causes of foodborne disease in the U.S. While these agents are responsible for diseases such as gastroenteritis and hepatitis, the true scope and significance of foodborne viral infection is drastically underestimated due to inadequacies in reporting and detection methods. The introduction of molecular biological techniques offers sensitive and specific alternatives for the detection of these previously non-detectable viral agents. The purpose of this research is to refine molecular methods to detect human enteric viruses from foods and to further develop approaches for the investigation of outbreaks of foodborne viral disease. The specific objectives are as follows: (1) Refine methods to extract human enteric viruses from foods, (2) Improve sensitivity, specificity, and speed of virus detection and confirmation, (3) Develop methods specifically for the detection and identification of small-round structured viruses of epidemiological significance to humans, and (4) Develop a comprehensive approach to the investigation of outbreaks of foodborne viral disease by linking detection and identification in clinical (fecal) and food specimens. The successful completion of this project will provide rapid and economical methods for the detection of viral contamination of foods and the investigation of foodborne viral disease outbreaks. These benefits will ultimately improve the safety of food products, protect public health, and minimize financial losses due to viral contamination of foods.

9802741 Stress-Induced Resistance to High Pressure in *Listeria monocytogenes* and *Escherichia coli* O157:H7

Yousef, A. E.; Shellhammer, T. S.
The Ohio State University; Department of Food Science and Technology; Columbus, OH 43210
Grant 98-35201-6212; $90,000; 2 Years

High pressure processing is a novel, non-thermal technique for inactivating pathogens in food by the application of extremely high pressures. Application of pressures in the range of 5000-9000 atmospheres for 1-5 minutes, at room temperature, can significantly reduce the microbial population in food and dramatically extend its shelf life. Since heat is not used in the process, negligible flavor and nutrient changes occur as a result of the high pressure treatment.

During the past two decades, new food-transmitted diseases emerged such as those caused by the enterohemorrhagic *E. coli* and the meningitis-causing *Listeria*. Such pathogenic bacteria are more likely to survive during food processing if they were exposed to conditions that make them resistant to preservation methods. For example, bacteria that are normally sensitive to heat may become heat-resistant when they are stressed during production, harvesting or even mild processing of food. Therefore, adaptation of bacteria to various stresses may compromise the safety of food. This challenge to the food industry needs urgent attention when new processing technologies (such high pressure processing) are introduced.

This project will identify the potential causes for increased resistance of pathogens to pressure. Additionally, the study will assess, in quantitative terms, the relationship between stress that foodborne pathogens may encounter and resulting stress-induced resistance to high-pressure processing. The outcome of this project will help food manufacturers develop strategies to overcome and eliminate stress-adaptation in foodborne pathogens.

9802822 The Molecular Epidemiology of *Clostridium perfringens* Type A Food Poisoning

McClane, B. A.
University of Pittsburgh School of Medicine; Department of Molecular Genetics and Biochemistry; Pittsburgh, PA 15261
Grant 98-35201-6349; $160,000; 2 Years

Clostridium perfringens type A food poisoning currently ranks as the second most common foodborne disease in the US. The diarrhetic and cramping symptoms of this illness are caused by *C. perfringens* enterotoxin (CPE). Recent studies have shown that the *CPE* gene encoding this enterotoxin can be located on either chromosomal or extrachromosomal DNA. However, only *C. perfringens* isolates carrying an extrachromosomal *CPE* can cause nonfoodborne intestinal disease. This project will evaluate four possible explanations for the strong association between chromosomal *CPE* isolates and food poisoning: i) isolates carrying a chromosomal *CPE* are predominant in the food poisoning environment, ii) chromosomal *CPE* isolates are more resistant to food-associated stresses (e.g. cooking) than are isolates carrying an extrachromosomal *CPE*, iii) the chromosomal *CPE* is more stable to food-related stress than the extrachromosomal *CPE*, and iv) food-related stress induces migration of the extrachromosomal *CPE* onto the chromosome. These studies should improve the safety of the American food supply by distinguishing whether only chromosomal *CPE* isolates are able to cause food poisoning, or if isolates carrying an extrachromosomal *CPE* can be converted, by food-related stress, into chromosomal *CPE* isolates. This information will improve the safety of the American Food supply in two ways: 1) it will become possible to specifically detect the presence of *C. perfringens* food poisoning isolates in foods before these foods are consumed, and ii) it will elucidate how/when *C. perfringens* food poisoning isolates enter foods, which will allow the development of strategies to interfere with the introduction of *C. perfringens* food poisoning isolates into foods.

9802728 Inactivation of Foodborne Pathogens Exposed to a Uniform Glow Discharge Plasma

Golden, D. A.; Montie, T. C.
University of Tennessee; Departments of Food Science and Technology and Microbiology; Knoxville, TN 37901
Grant 98-35201-6270; $71,442; 1 Year

Increased emphasis on food safety has intensified research efforts to develop and evaluate new and innovative means of inhibiting, destroying, and controlling pathogenic microorganisms in foods. The overall objective of this research is to evaluate the efficacy of a One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) for its ability to destroy foodborne pathogens. The OAUGDP is a newly-invented form of electron discharge which generates a uniform glow discharge plasma (plasma - the fourth state of matter) in atmospheres of various gases such as helium, carbon dioxide, and most importantly, air. The sterilization properties of the OAUGDP include ozone and other oxidative species, ultraviolet photons, photons in the visible part of the spectrum, charged particles, and neutral particles. Specific objectives of our proposed work are 1) to determine the susceptibility of ten foodborne pathogenic microorganisms to inactivation upon exposure to the OAUGDP, and 2) to determine the effect of culture age, pH, and growth temperature on the susceptibility of these foodborne pathogens to inactivation. Our previous studies have shown that the OAUGDP is an effective means of destroying various microorganisms. Furthermore, our results indicate that treatment of culture media with the OAUGDP for the times required to kill bacteria does not result in the development of by-products toxic to microbial growth. The OAUGDP unit has the potential to be adapted as an in-line process suitable for application as a mechanism of pasteurizing foods and controlling foodborne pathogens.

9802844 Sporulation Control of Enterotoxin Synthesis in *Clostridium perfringens*

Melville, S. B.
University of Tennessee; Memphis; Department of Microbiology and Immunology; Memphis, TN 38163
Grant 98-35201-3496; $130,000; 2 Years

Clostridium perfringens is a common source of food poisoning in humans, responsible for 10% of the outbreaks in the U.S. Most large outbreaks of *C. perfringens* food poisoning are associated with commercial food services, such as restaurants and institutions, but many infections occur in the home as well. The symptoms of the disease (diarrhea, nausea and vomiting) are due to the production of a potent enterotoxin protein (CPE) in the intestinal tract by sporulating bacteria. The ability of *C. perfringens* to produce a heat resistant spore not only leads directly to the production of the enterotoxin, but also leads to increased outbreaks of the disease. Often, foods are prepared at high enough temperatures to kill vegetative cells, but not spores. If the food is not refrigerated, the spores germinate and the cells grow rapidly. When the contaminated food is eaten, the cells sporulate in the small intestine, releasing the enterotoxin. We are interested in determining how the sporulation process regulates *cpe* gene expression at the transcriptional level. To study this, we will take two experimental approaches: (1) Purify RNA polymerase enzymes that transcribe *cpe* gene promoters (2) Determine in which cell compartment of the developing spore transcription factors needed for *cpe* gene expression are made. Together, these studies will contribute to our knowledge of how heat resistant spores and enterotoxin are produced by the cell. This information can then be used to develop better food handling procedures to reduce the incidence of this very common disease.

9802799 Novel Antimicrobial Systems for Control of Foodborne Pathogens

Johnson, E. A.
University of Wisconsin; Department of Food Microbiology and Toxicology; Madison, WI 53706-1187
Grant 98-35201-6214; $90,000; 2 Years

The microbiological safety of imported and domestic fruits, vegetables , and certain other foods has become a major priority of the USDA, FDA, and HHS. Recent studies from our laboratory have demonstrated that certain food-approved flavorants markedly sensitize foodborne pathogens including *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* to various classes of food-grade antimicrobials and to certain antibiotics. In particular, in the presence of low concentrations (< 100 ppm) of the flavorants nerolidol and farnesol, microoorganisms are inhibited by markedly lower doses of certain antimicrobial agents and antibiotics. These compounds are derived from natural plant sources. Our results indicate that these and related compounds could be used to increase the efficacy of other food-grade antimicrobial agents. In the present study, sensitization by terpenoids and inactivation of foodborne pathogens will be investigated. Gram-negative and gram-positive bacterial pathogens will be exposed to potential sensitizing agents, and then evaluated for extent and kinetics of inactivation by sanitizers, food-grade antimicrobials, and antibiotics. Killing will be assayed by traditional plating, and by flow cytometry. Organisms to be tested include strains of *E. coli* O157:H7, *Salmonella* serovars, *Listeria monocytogenes*, and *Clostridium botulinum*. The successful completion of this proposal will provide novel elimination and sanitation technologies to reduce the risk of foodborne disease from foods and food contact surfaces and will enhance the public and media image regarding the microbiological safety of raw and minimally processed foods.

9802828 DNA-Binding Proteins CspE and Dps Protect DNA at Low pH in *Escherichia coli* O157:H7

Kaspar, C. W.
University of Wisconsin; Department of Food Microbiology and Toxicology; Madison, WI 53706
Grant 98-35201-6213; $115,000; 2 Years

An important characteristic of *Escherichia coli* O157:H7 is the ability of 10 to 100 O157:H7/gram of raw ground beef to survive processing, storage, cooking, and host-defense systems and cause illness. It is hypothesized that acid tolerance is a contributing factor to the low-infectious dose noted for this human pathogen. Sustained acid tolerance in serotype O157:H7 strains is primarily regulated by the stationary-phase sigma factor σ-^{38}, encoded by *rpoS*. We have identified a *rpoS*-regulated Protein (CspE) that is present in acid tolerant strains of serotype O157:H7 strains and absent, or present at reduced quantities, in acid-sensitive strains. This protein has been previously identified as a cold-shock protein with no known function. Analysis of protein and DNA sequences finds a high proportion of basic amino acids, indicating that CspE is a DNA-bind protein. Dps is another previously described *rpoS*-regulated protein with DNA-binding properties that result in DNA protection against oxidative stress. Because DNA is sensitive to low pH and survival is dependent upon its protection, the goal of this project is to define the contributions of CspE and Dps to DNA protection in low pH environments (i.e. foods and synthetic gastric fluid). Results from this study will provide industry and public health agencies with the much needed data to further refine strategies for control, identify areas of risk, and add to the scientific knowledge on the survival of *E. coli* O157:H7.