Annual Report 1999-2000

The Joint Institute for Food Safety and Applied Nutrition

Executive Summary

In 1996, the Commissioner of the Food and Drug Administration, Dr. David Kessler, and the President of the University of Maryland, Dr. William Kirwan, met to discuss opportunities for cooperative interactions that would be productive for both institutions. The result of this and a number of subsequent meetings was the April 15, 1996 signing of a Memorandum of Understanding (MOU) that established a cooperative venture, the Joint Institute for Food Safety and Applied Nutrition (JIFSAN). JIFSAN was initially established as a cooperative venture between the University and the FDA Center for Food Safety and Applied Nutrition (CFSAN). Later, the MOU was amended to include the FDA Center for Veterinary Medicine (CVM).

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is a jointly-administered multidisciplinary research and education program. Dr. David R. Lineback (University of Maryland) is the Director and Dr. Samuel W. Page (FDA) and Dr. Paul Mazzocchi (University of Maryland) are Associate Directors. Dr. Norris Alderson is the CVM representative. FDA's broad goals within the collaboration are to expand food safety, human nutrition, and animal health sciences research and education programs that are necessary to provide the Agency with expertise and knowledge needed to recognize and effectively deal with emerging food safety issues before they become crises and to enhance regulatory review capabilities. Collaborative activities involve research, education, and outreach in four major areas: risk analysis, microbial pathogens and toxins, food constituents and applied nutrition, and animal health sciences: food safety.

The new CFSAN office and laboratory building will be adjacent to the University of Maryland in College Park enabling FDA and the University to share many resources, such as major instrumentation and library facilities. JIFSAN provides a neutral environment in which experts from industry, consumer and trade groups, international organizations, government agencies, and academia pool their resources and ideas to provide the scientific base for the development of sound public health policy. Members of the JIFSAN Advisory Council provide advice, vision, and support critical to advancing JIFSAN's mission of cooperative research and education/outreach. Visiting scientists are encouraged from all sectors. The interactions of FDA, the University, and visiting scientists help ensure that regulatory personnel remain in the forefront of food safety issues. This also provides visiting scientists insight into regulatory processes. Opportunities for undergraduate and graduate students to work with FDA scientists enhance students' understanding of regulatory processes and will provide them with valuable practical experience. Programs initiated by JIFSAN have demonstrated that the benefits to be achieved by this partnership are substantial. Collaborative research

projects contribute to the science undergirding current and future regulatory issues and activities that impact on public health policies.

Risk analysis (risk assessment, management, and communication) is a major focus of JIFSAN programs. This effort promotes the development of risk-based, scientifically-supportable safety standards. These standards can deliver the intended degree of measurable public health protection and can be used to identify priorities to effectively apply available resources. JIFSAN is developing new approaches to information management related to risk analysis. With oversight from the interagency Risk Assessment Consortium (RAC) in the Food Safety Initiative, a Food Safety Risk Analysis Clearinghouse is being developed at JIFSAN. This provides a mechanism to collect and disseminate available data and methodologies from government, academia, and industry. The intent of the Clearinghouse is to provide a centralized information source in areas of risk assessment related to food safety with initial emphasis on microbial pathogens and their toxins. The unique feature of this clearinghouse model lies in the examination and documentation of state-of-the-art methods, data sources, and current results of on-going risk assessments so that a much more complete and up-to-date picture of risk assessment is assembled.

The development of partnerships with external constituencies is one of the major avenues JIFSAN uses to expand the science base available for addressing public health policy issues. University and FDA scientists have begun collaborative research efforts with other organizations.

An internal collaborative research program provides seed funding to University of Maryland faculty to support research projects that are closely aligned with FDA's research needs. FDA collaborators on each project help provide additional scientific expertise and insight into the public health impact of the research.

Trade initiatives have put food safety high on the international agenda. JIFSAN is actively involved in developing collaborations with international organizations to facilitate cooperative research and education programs and the exchange of scientists. In addition, JIFSAN has been designated a Pan American Health Organization/World Health Organization Food Safety Collaborating Center that focuses on risk assessment of contaminants in foods and mycotoxin analysis.

The MOU established a set of relationships that closely link the University with CFSAN and CVM by committing to the sharing of facilities, personnel, and intellectual resources when appropriate. Thus, FDA personnel will have access to University facilities such as libraries and may be appointed as adjunct or research faculty in recognition of their involvement in cooperative programs in research, teaching, mentoring, and direction at the graduate and undergraduate levels. FDA will support and utilize major instrumentation facilities (electron microscopy, nuclear magnetic resonance spectroscopy) on the campus and those facilities will house appropriate University of Maryland and FDA personnel. These and other synergistic relationships outlined in the

MOU will allow both institutions to remain state of the art in a number of areas where duplicative efforts would be less than successful.

Subsequent to the signing of the MOU, FDA and University personnel developed an Umbrella Cooperative Research and Development Agreement (CRADA) and a multi-party CRADA template. These tools were implemented or designed to facilitate the development of collaborative research beyond the internal competitive research program described later and to provide a mechanism to address issues related to shared resources.

Support for the operation of JIFSAN was provided by FDA and the University. FDA provided a cooperative agreement for $6.5M for five years starting on September 30, 1997. The University provided support in several ways including the return of 100% of the Designated Research Initiative Fund (DRIF) funds from the cooperative agreement to JIFSAN, providing space and administrative support to the program in the form of personnel, and providing space for instrumentation facilities.

The actual operation of JIFSAN commenced with its initial funding on September 30, 1997. Dr. Paul Mazzocchi, Dean of the College of Life Sciences, as Principal Investigator on the FDA-JIFSAN cooperative agreement, served as Acting Director of JIFSAN while the search for the permanent Director was conducted. Dr. David Lineback became Director in November 1998.

Progress Report

During the third year of operation for JIFSAN, the administrative structure was strengthened, several education and outreach programs were developed and accomplished, research programs were continued and new ones initiated, and contacts were developed to build partnerships/strategic alliances to plan and initiate additional research, education, and outreach programs. Progress in these areas will be outlined with specific examples included.

The non-competitive base funding for the third year was $2,047,840. This was increased to $2,747,823 in response to a Federal Register Notice (64 FR 40380) indicating FDA's intention to supplement the cooperative agreement up to an estimated $2 million per annum to the original award amount. These additional funds will provide support to JIFSAN for the purpose of addressing emerging health issues and crises that are related to food safety and the development and evaluation of risk communication and management paradigms. The additional funds awarded September 30, 1999 were used for the initiation of a cosmetics research program; support for the development of training courses on food safety issues; and training in international Good Agricultural Practices. In August 2000, the cooperative agreement was supplemented to support the development and piloting of a model interactive web-based Adverse Event Reporting System, and additional support for a conference on Valuing the Benefits of Food Safety.

Administrative Structure

A unique administrative structure is needed for JIFSAN to allow it to most effectively use resources while planning, organizing, and accomplishing multidisciplinary, multi-institutional programs in research, education, and outreach. An effective way to do this is to utilize, to the greatest extent possible, the administrative structures available in the University of Maryland as one of the major partners in JIFSAN. The structure and policies of a major land-grant university offer the flexibility needed to enable JIFSAN to create and operate strategic alliances involving multiple partners and multiple funding sources.

Specific progress in strengthening JIFSAN's administrative structure includes:

- Ms. Judy Dillon began work as Administrative Assistant on February 15, 2000 following the departure of Ms. Shelia Richburg.

- Two additional offices were obtained, on a permanent basis, for use by JIFSAN. One of the offices is used by FDA staff when work necessitates their being at the University of Maryland. This office will be used for the Conference and Communications Coordinator, a position that will be added in early 2001. The second office is currently used as a small conference room for JIFSAN meetings, particularly the Working Group meetings.

- Both office space and computer laboratory space (approximately 500 sq. ft.) is utilized by the JIFSAN Food Safety Risk Analysis Clearinghouse operations at the VA-MD Regional College of Veterinary Medicine at College Park. This space is used to develop and operate the Clearinghouse and to support risk analysis activities.

- The FDA JIFSAN Liaison Staff was formalized by assigning two individuals within CFSAN to Associate Director Samuel Page. They are working with Dr. Page on JIFSAN activities within the FDA. This Liaison Staff and their responsibilities are described in a document entitled JIFSAN Program Management and Coordination Activities.

- An initial draft of a strategic plan for JIFSAN was developed. Four task groups were involved in developing a strategic plan for the four areas of risk analysis; animal health sciences: food safety; food constituents and applied nutrition; and microbial pathogens and toxins. The previous mission statement was revised and a vision was crafted by the JIFSAN Working Group. These were shared with the JIFSAN Advisory Council, FDA staff, and University of Maryland personnel. Their comments will be discussed and incorporated during further development of the plan. Implementation of the strategic plan will be initiated in 2001.

The JIFSAN Advisory Council

Central to the operation of JIFSAN is an Advisory Council composed of members from private sector business, government agencies, academia, and representatives of consumers' interests. This group will provide guidance to JIFSAN in developing research, education, and outreach programs to address problems in food safety, nutrition, animal health sciences, and risk analysis. Organization of the Council continued with its second meeting held on September 27-28, 2000. Progress of JIFSAN programs and the draft strategic plan were the major foci of the meeting. Numerous comments and suggestions were presented for consideration by JIFSAN in further development and implementation of the strategic plan.

Members of the Advisory Council currently include:

- Private sector industry

  Bestfoods (Ms. Diani Santucci)
  Coca-Cola Company (Dr. Michael Carakostas)
  Campbell Soup Company (Dr. George Evancho)
  Dean Foods Company (Dr. George Muck)
  Frito-Lay (Dr. Steve Saunders)
  General Mills (Mr. Frederick Hegele)
  Gerber Products Company (Dr. Nicholas Hether)
  Hershey Foods Corporation (Dr. Stanley Tarka)
  Kellogg Company (Dr. Tracie Sheehan)
  Kraft Foods (Mr. Ron Triani)
  McCormick and Company (Dr. Hamed Faridi)
  McNeil Specialty Products Company (Dr. Steven Mann)
  M&M/Mars (Dr. Steven Rizk)
  Mead Johnson Nutritionals (Dr. Thomas Ferguson)
  Monsanto Company (Dr. Jerry Hjelle)
  Nabisco (Dr. W. Kelly Jones)
  Ocean Spray Cranberries (Dr. Y. Steve Henig)
  Odwalla (Mr. Stephen Williamson)
  Procter and Gamble Company (Dr. Keith Triebwasser)
  Tropicana Products (Dr. Nancy Green)

- Representatives of Consumers' Interests

  Ms. Carol Tucker Foreman (Consumer Federation of America)
  Ms. Laurie Girand (Safe Tables Our Priority)
  Ms. Linda Golodner (National Consumers League)
  Dr. Kristen McNutt

- Academia

  Dr. Lester Crawford (Center for Food and Nutrition Policy,
  Georgetown University)

Dr. Michael Doyle (University of Georgia)
Dr. Julie Miller Jones (College of St. Catherines)
Dr. Sanford Miller (Center for Food and Nutrition Policy,
Georgetown University)
Dr. Michael Pariza (University of Wisconsin)
Dr. Stephen Taylor (University of Nebraska)
Dr. Connie Weaver (Purdue University)

- Government

  Dr. Peter Stanley (Central Science Laboratory, MAFF, UK)

- Individuals

  Dr. Gilbert Leveille (McNeil Consumer Healthcare)

Development of the Advisory Council will continue next year with inclusion of additional members from private sector business, academia, government agencies, and representatives of consumers' interests

Research Initiatives

Research is a major focus of JIFSAN. Collaborative research supports the goal to develop a strong science base to address ongoing and increasingly complex public food safety issues.

JIFSAN is involved in research in a number of ways:

- By supporting core facilities that benefit FDA and University scientists and their collaborators,
- By funding a competitive internal research program through the Cooperative Agreement,
- By developing cooperative programs with external constituencies.
- By facilitating programs funded from multiple institutions and other granting sources.
- By supporting scientists working on JIFSAN programs.

By the very nature of the research enterprise, i.e. the time required for building functioning multidisciplinary collaborative research teams and the highly competitive nature of obtaining external research funding, establishing externally-funded research programs for JIFSAN is still in the development stage and will require time.

JIFSAN Competitive Internal Research Program:

A collaborative research program was established in 1998 by providing seed funding of $25,000 each for four research projects. Each project requires collaboration between at least one University of Maryland faculty member and one or more FDA collaborators. The latter help provide additional scientific expertise and insight into the public health impact of the stated research. These projects contribute to the science for current and future regulatory issues and activities that impact on public health policies, and are aligned with the FDA's research needs:

- Development of sampling and detection methods for the identification of pathogens and toxins.
- Further develop an understanding of antibiotic drug resistance.
- Development of methods to characterize the composition and bioactivities of natural constituents of foods, including micronutrients and beneficial non-nutrients.
- Developing messages pertaining to food safety and the adoption of safe food handling practices.

The system for funding grants in the internal research program was standardized to be support for a graduate student plus operational support (a total of $30,000) or for a postdoctoral associate plus operational support (a total of $40,000). Operational support is $10,000 in each case. Proposals may be for three years, but are funded for only one year at a time. Continuation is contingent upon a satisfactory annual progress report, a proposal for continuation of the research, and availability of funding.

The first four projects funded in May 1998 were reviewed on the basis of a progress report and a proposal for continuation of the research. All four were funded for a third (final) year. In addition, five projects were funded in January 1999. Two of these were proposed for three years, one for two years, and two for one year only. Progress on the three multiple-year projects was reviewed and they were continued for a second year. Five projects, one of which was for a single year and one was for two years**,** were funded in January 2000.

Third (final) year projects:

- **Immunoaffinity Hollow Fiber Ultrafiltration for High Throughput Screening/Residue Analysis in Food Safety.** Cheng Lee (UM) and Mary Trucksess (FDA).

  Modern agriculture has benefited from the development and use of agrochemicals including insecticides, herbicides, and veterinary drugs. In addition to these exogenous chemicals, many undesirable compounds such as plant toxicants and mycotoxins are known to occur naturally. Thus, there

exist critical needs for monitoring residue levels in foods, agricultural commodities, and environmental samples. To perform rapid and sensitive residue screening and confirmation in food safety, advanced bioanalytical techniques for multiresidue analysis of parent compounds and their metabolites are essential.

The proposed immunoaffinity ultrafiltration combines the strengths of membrane filtration in the ease and speed of separation and concentration with the specificity of antigen-antibody interactions. The food contaminants and residues are selected from their sample matrices by formation in solution of noncovalent immunoaffinity complexes with antibodies raised against targeted compounds. Captured compounds in immunoaffinity complexes are retained and separated from other sample components using membrane ultrafiltration based on their differences in size. The specifically selected compounds retained in membrane ultrafiltration are subsequently liberated from the antibodies by acidification or organic solvent for on-line interfacing with electrospray ionization mass spectrometry (ESI-MS).

A microdialysis junction, based on dialysis tubing, serves as liquid junction between the outlet of membrane ultrafiltration and ESI-MS. The junction not only introduces an acidic solution containing organic solvent for antibody dissociation but also defines the electrical voltage needed for the electrospray ionization of food contaminants and residues. In addition to the rapid and sensitive identification of analytes, MS detection can potentially provide structural analysis of targeted compounds in a MS/MS mode. Multiresidue screening and analysis of veterinary drugs, pesticides, toxicants, and mycotoxins can be established by employing multiple antibodies and immunoaffinity complexes in a single membrane ultrafiltration. Each ultrafiltration membrane acts as an immunoaffinity extraction of targeted compounds and elutes the analytes for structural determination and elucidation using ESI-MS.

Based on the experience in development of an immunoaffinity hollow fiber ultrafiltration system, a miniaturized immunoaffinity ultrafiltration system in a single platform was constructed using micromachining techniques for significant reduction in both dead volumes and sample consumption. Two microdialysis membranes are sandwiched between three plastic substrates micromachined with four serpentine flow channels. Samples containing the reaction mixture of targeted compounds and antibodies are infused through the sample inlet in the second substrate. During operation, unbound compounds diffuse through the dialysis membrane and elute from the dialysis buffer outlet in the first substrate. Captured compounds are retained in the second substrate and flow through the hole drilled through the substrate into the second microdialysis stage for concentration and enrichment. The solution containing immunoaffinity complexes enters the second microdialysis stage and is exposed to a counter-current flow of dry air for

water evaporation and analyte concentration. The concentrated complexes then flow directly to the microdialysis junction for complex dissociation and ESI-MS detection.

The microchannels on the plastic substrates are fabricated using the template imprinting technique. The dimensions (100 μm in width, 30 μm in depth, and 16 cm in length) of imprinted microchannels on the copolyester substrates are examined using an optical profilometer. By fixing the flow rate of dialysis buffer at 30 μl/min, the dialysis efficiency is dependent on the residence time of analytes in the microchannel and, thus, the sample flow rate. In the absence of antibody raised against aflatoxin B_1, a control experiment is carried out to ensure that all aflatoxin compounds have sufficient time to diffuse across the membrane and be carried away by fresh dialysis buffer. The flow rate has to be at or lower than 100 nl/min in order to completely remove all aflatoxin compounds.

The antibody employed in this study is raised against aflatoxin B_1 and cross-reacts with the other aflatoxin compounds (except aflatoxin G_{2a}) examined in this study. The molecular weights and corresponding $(M+H)^+$ ions of aflatoxins in the positive electrospray ionization mode are summarized as: aflatoxin B_1 (MW 312.3, M+ H 313.3), aflatoxin B_2 (MW 314.3, M+ H 315.3), aflatoxin G_1 (MW 328.3, M+ H 329.3), aflatoxin G_2 (MW 330.3, M+ H 331.3), aflatoxin G_{2a} (MW 346.3, M+ H 347.3). The reaction mixture is prepared in a 10mM phosphate buffer at pH 7.4 with final concentrations of 5 x 10^{-7} M and 1 x 10^{-6} M for each aflatoxin and antibody, respectively. At a 4:1 molar binding ratio of antibody to each aflatoxin, all aflatoxins except aflatoxin G_{2a} are retained during membrane ultrafiltration by the formation of aflatoxin-antibody complexes and detected using ESI-MS. To demonstrate the screening power of immunoaffinity ultrafiltration, urine controls are purchased from Bio-Rad and are added with reaction mixture containing aflatoxins and antibody. No additional m/z ions, other than the ones corresponding to aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2, are detected by scanning between m/z 100 and m/z 700 in ESI-MS. All low molecular-weight urine components are completely removed during the membrane dialysis process.

To investigate the competitive binding of aflatoxins toward antibody, the molar binding ratio of antibody to each aflatoxin is reduced from 4:1 to 1:4 by decreasing the antibody concentration from 1 x 10^{-6} M to 6.25 x 10^{-8} M. Only aflatoxin B_1, which exhibits the strongest binding strength toward antibody among the aflatoxins, can be detected using ESI-MS. Due to the lower complex (aflatoxin B_1-antibody) concentration, the ion intensity observed for retained aflatoxin B_1 is therefore smaller.

By assuming the complex concentration of 1.25 x 10^{-7} M at a sample flow rate of 100 nl/min, the retained aflatoxin B_1 measured during the 5-sec mass scan

is estimated to be around 0.3 pg. Our detection sensitivity is at least one to two orders of magnitude better than those reported in the literature. Significant enhancement in detection sensitivity is mainly contributed by minimized sample loss and use of the concentration unit in the miniaturized immunoaffinity ultrafiltration system. The combination of "nanoscale" sample manipulation in microfabricated devices using ESI-MS detection is particularly attractive due to the speed and sensitivity that can be achieved with MS, as well as the amenability of ESI-MS to the low flow rates. Furthermore, a microfabricated device provides a major step toward the development of a compact, robust, and automated immunoaffinity ultrafiltration platform for multiresidue screening and analysis in support of food safety.

Publication status:

1. Jiang, Y., Lee, C.S., "Immunoaffinity Ultrafiltration Coupled with Electrospray Ionization Mass Spectrometry for High Throughput Residue Analysis," submitted to Journal of Mass Spectrometry.

2. Jiang, Y., Gao, J., Lee, C. S., "Miniaturized Immunoaffinity Ultrafiltration Coupled with Electrospray Ionization Mass Spectrometry for Mycotoxin Analysis," in preparation for Analytical Chemistry.

- **Effect of a Variety of Stress Factors on the Immune Systems of Poultry and Subsequent Infection of Shell Eggs by Salmonella.** Wenxia Song (UM) and Richard Raybourne (FDA).

  Salmonella enteritidis (SE) carried by chickens and shell eggs has become a major source of human intestinal infections. Despite the tremendous efforts made by the poultry industry, no effective measurements for elimination of SE-colonization have been generated. Our goal is to characterize the immune responses of hens against SE, and identify variables, especially stress conditions, affecting hen's immunity and promoting SE spread. We have analyzed the colonization process of orally inoculated SE, and SE-induced immune responses in young chicks. The effect of an immunosuppressing virus on chicks' immune response against SE-infection was also examined.

  Through the oral route, we successfully inoculated one-day old chicks with an egg isolate of SE. The chicks remained infected for at least eight weeks after the inoculation. However, the SE-shedding is significantly decreased by the eighth week. Histological analysis showed mild pathological lesions in SE-infected chicks, characterized by necrosis of villous epithelium and heterophilic infiltration in the colonizing sites of intestine. Coinfecting chicks with both SE and a common immunosuppressing virus, chicken anemia virus (CAV), had no significant effect on the bacterial colonization and shedding.

Using SE that was modified to express the green fluorescence protein (GFP-SE) and immunohistochemistry, the colonization process of orally inoculated SE in young chicks was followed. Two weeks after inoculation, SE was primarily located in the epithelial surface of the intestine. Macrophage-like cells that contained GFP in their cytoplasm were found in the lamina propria and the core of villi, suggesting that these cells engulfed and digested GFP-SE releasing GFP in their cytoplasm. By the fourth week, the number of GFP-positive macrophage-like cells decreased, and GFP-SE was detected both within the host cells and in the lumen of the intestine. By the eighth week, most of the SE resided inside host cells. This indicates that, with time, SE gradually moved from the epithelial surface to the inside of host cells, which is consistent with the data from our bacteriology study that infected chicks shed less SE, but remained SE-positive by the eighth week. Our studies describe the SE-infection process, from the colonization on epithelial surface to the intracellular replication stage within eight weeks of time, in young chicks. This finding is especially important because *Salmonella* is known as an intracellular pathogen and escapes the host immune system by hiding in host cells.

Next, we analyzed the immune response of chicks induced by SE-infection. To evaluate the mucosal immunity, the concentration of mucosal IgA and the number of cells secreting or carrying IgA were determined. We found that the concentration of SE-specific IgA in the intestinal mucosa of infected chicks increased with time, and was 10 times that seen in uninfected chicks by the eighth week. The number of IgA-positive cells in the intestine of infected chicks was also significantly higher than that seen in uninfected chicks. When chicks were inoculated with both SE and CAV, the concentration of SE-specific IgA in the intestinal mucosa was significantly lower than that seen in chicks infected with only SE. These data suggest that the oral SE-infection stimulates mucosal humoral immunity specific for SE, and the immunosuppressing virus, CAV, inhibits the immune response. The central humoral immunity was evaluated by the concentration of SE-specific IgG in sera and the number of germinal centers in spleens. We found a higher concentration of SE-specific IgG in sera and a greater number of germinal centers in spleen of infected chicks compared to uninfected chicks. This indicates that the central humoral immunity is also stimulated by SE-infection.

Altogether, orally inoculated SE initially colonizes on the epithelial surface of chick's intestine and enters host cells over 4-8 weeks time. In the early stage of the infection, macrophages appear to participate in the clearance of SE-infection by engulfing and digesting the bacteria. The colonization of SE stimulates both mucosal and central humoral immune responses specific for SE. When the concentrations of SE-specific IgA and IgG were increased, SE-shedding was decreased, suggesting that humoral immune responses play a role in clearing SE-colonization in the lumen of intestine. But the intracellular

SE remained, suggesting that their intracellular location helps them to escape from host immunity.

- **Surveillance of Poultry and Other Stock for Carriage of Multiresistant Enterococcus.** Lewis Carr, Sam Joseph (UM) and David Wagner (FDA).

  SPECIFIC AIM #1: Environmental surveillance of poultry for *Enterococcus* spp. and determination of resistance.

  Of the more than 1000 bacterial isolates recovered from over 70 commercial broiler and roaster poultry farms of the Delmarva Peninsula, 541 *Enterococcus* isolates have been identified and confirmed during year one of funding. Subsequent isolates are still under investigation and of the roughly 400 isolates, 105 presumptive *Enteroccus* spp. have been isolated from more than 80 farms. These isolates have come from swabs of fecal material found on poultry transport containers (PTCs) as well as litter from farms. A full protocol for authoritative identification to species has been developed and is currently being applied to all isolates.

  Using this identification schema, we have found ~60% of our isolates to be *E. faecalis*. *E. faecium* was the next most common isolate (20%). The *E. faecium* designation of four biotypes is on the basis of biochemical reaction differences for the fermentation of the carbohydrates raffinose and sucrose. Other isolates of note include those that fall into one of the five groups (I-V) which were not definitively identified to species due to the substantial, but not unexpected, deviation from established biochemical profiles derived from established species.

  Antimicrobial susceptibility testing for all 541 isolates from year one is almost complete and testing of year two isolates is about to begin. Uniform resistance to low levels of aminoglycosides (amikacin, apramycin, gentamicin, kanamycin, and streptomycin) and all classes of cephalosporins (ceftiofur, ceftriaxone, cefazolin and cephalothin) is apparent in all species of *Enterococcus*. Examining resistance of the two species that constitute greater than 80% of the isolates (*E. faecalis, E. faecium*), differences in resistance appear in nitrofurantoin (1.8%, 84.5%), penicillin (0.7%, 73.2%), and the lincosamide clindamycin (2.1%, 56.3%). Resistance to tetracycline was high in both species with 95%-75% of isolates resistant. Macrolide resistance (erythromycin, clarithromycin) was consistent within the species with higher rates found in *E. faecalis* (69.5%) than in *E. faecium* (36.7%). Resistance to the flouroquinolones ciprofloxacin and lomefloxacin was consistent within *E. faecium* (88.7%, 86%) but not *E. faecalis* (25.3%, 100%). Synercid resistance was found to be remarkably high at 60.9% of all *E. faecium* isolates. High-level aminoglycoside resistance was evident in both species with high-level streptomycin resistance found in 39.5% and 25% of isolates, while gentamicin resistance was seen at relatively low levels in *E. faecalis* (5.6%) and not

detected in *E. faecium*. No unexpected vancomycin resistance has been detected. The high level of resistance in this organism suggests that this reservoir of resistance may compromise the therapeutic potential of quinupristin-dalfopristin for humans.

In an effort to expedite the workload, the Vitek® identification system has been incorporated to verify the grouping patterns of new isolates into manageable classes. Recently reported molecular methods that have proven to be more discriminatory and consistent will be employed to further refine this categorization in the near future. Selected isolates (n=179) have been screened against a new panel of antibiotics which include the production antibiotics bacitracin, flavomycin, salinomycin, tylosin, lincomycin, and virginiamycin. The panel also extends the range of the antibiotics vancomycin, erythromycin, penicillin, tetracycline, and gentamicin and adds kanamycin and streptomycin at high concentrations.

Analysis of the resistance patterns of *E. faecium* to this new panel reveals several important details. Briefly, resistance to these production drugs was prevalent, with resistance rates of 79%, 71%, 35%, and 35% for penicillin, tetracycline, erythromycin, and high-level streptomycin, respectively. Resistance to bacitracin (99%), flavomycin (97%), lincomycin (93%), and tylosin (23%) was observed at levels of 256, 32, 32, and 32 μg/ml, respectively. No high-level gentamicin or vancomycin resistance was observed, although resistance to streptogramin, virginiamycin (62.5% at ≤4 μg/ml), corresponded closely with resistance to the human analogue, quinupristin-dalfopristin (60%). The resistance profile of this collection of *E. faecium* isolates that originate from the commercial production environment suggests that resistance develops and predominates with the use of antimicrobials in the production environment and may mirror the extent of use within the region. While debate continues on the origin of *E. faecium* resistance from the human population, these data support the assertion that agricultural usage of antimicrobial agents selects for resistance determinants that could exacerbate the resistance problem of enterococci from the human microbiota.

Newer isolates will be routinely screened against this panel in addition to the panel containing clinically applied gram-positive antimicrobials.

SPECIFIC AIM #2: Analysis of relatedness of resistant isolates

Comparative ribotype analysis of 53 human and 132 poultry isolates was done using the Qualicon RiboPrinter™. Human isolates were obtained from multiple medical centers across the U.S. and poultry isolates were collected from the Delaware-Maryland-Virginia peninsula. RiboPrint™ patterns from both human and poultry sources showed extensive diversity and several large clusters of strains. Interestingly, the *E. faecium* populations within humans

and poultry were nearly distinct. One poultry isolate fell in a large cluster of human isolates and one small cluster contained one human and one poultry isolate. While these data suggest that *E. faecium* isolated from poultry are unlikely to be the source of human clinical enterococcal isolates, more analysis is needed with a larger group of isolates.

A commitment of time and resources by the Center for Veterinary Medicine has been made to evaluate clonality of isolates using pulsed-field gel electrophoresis (PFGE) during the final year of funding.

SPECIFIC AIM #3: Viability of *Enterococcus* spp. in the feed production process

A protocol to monitor the viability of *Enterococcus faecium* throughout the feed production process has been proposed. In preparation, nine isolates of *Enterococcus* spp. have been isolated and characterized from feed that did not contain growth sub–therapeutic antibiotics. Generally, these isolates are more sensitive to antimicrobial agents, but high-level aminoglycoside resistance has been observed in two isolates.

Delays encountered include time constraints of researchers to address the efforts proposed. New equipment will facilitate standardized work throughput.

Comments:

The data generated to date demonstrate that *Enterococcus* isolates that have been recovered from the poultry environment possess broad resistance to antimicrobial agents. Plans are to perform pulse field gel electrophoresis studies of resistant isolates in the near future to support population studies of resistance in *Enterococcus* in addition to the surveillance of targeted resistance genes within sub-populations of the genus. In accordance with the goal to establish a diverse culture collection of *Enterococcus* spp., isolates from a variety of sources have been collected and characterized: 80 human isolates of *Enterococcus* spp. have been supplied by a Utah area hospital, ten of over 200 isolates from seagulls of the Pacific Northwest, more than 50 from different retail meat classes, and over 80 from a poultry production environment devoid of growth-promoting antimicrobials save the flouroquinolone, sarafloxicin. The analysis of this tremendous volume of data is incomplete, but ongoing. Notably striking are differences in antimicrobial susceptibility patterns of isolates of different ecological origin.

Publication status:

a. Presentations

- Joseph, S. W. "Association of Antibiotic Resistant Enterococci with Poultry," JIFSAN Advisory Council, College Park, MD, October 21, 1999.

- Hayes, J. R., D. D. Wagner, L. L. English, L. E. Carr, and S. W. Joseph. "Detection of Multiresistant Enterococci in the Poultry Environment," SPSS/International Poultry Exhibition, Atlanta, GA, January 17, 2000.

- Joseph, S. W. "Implications of Multiresistant Enterococci in Poultry," Center for Food Safety, Quality, and Environment, University of Georgia, Athens, GA, March 7, 2000.

- English, L. L., J. R. Hayes, D. G. White, S. W. Joseph, L. E. Carr and D. D. Wagner. "Antibiotic Susceptibility Profiles of *Enterococcus* Isolates from the Poultry Production Environment," FDA Science Forum – FDA and the Science of Safety: New Perspectives, Washington, D.C., February 2000.

- Joseph, S. W. "Antibiotic Resistance in *Enterococcus* spp. in Agriculture, CSL/JIFSAN Joint Symposium on Food Safety and Nutrition: Risk Assessment and Communication in Food Safety, York, UK, June 21, 2000.

b. Manuscripts

- Hayes, J. R., A. C. McIntosh, S. Qaiyumi, J. A. Johnson, L. L. English, L. E. Carr, D.D. Wagner and S. W. Joseph. 2000. High Frequency Recovery of Quinupristin-Dalfopristin (Synercid®) Resistant *Enterococcus Faecium* Isolates from the Poultry Production Environment. Journal of Clinical Microbiology (in peer review).

- Joseph, S. W., J. R. Hayes, L. L. English, L. E. Carr and D. D. Wagner. 2000. Implications of Multiple Resistant *Enterococci* Associated with the Poultry Environment. Journal of Food Additives and Contamination (in peer review).

- Hayes, J. R., D. D. Wagner, L. E. Carr, L. L. English and S. W. Joseph. 2001. A Two-year Surveillance of *Enterococci* Isolated from the Poultry Environment for Antibiotic Resistance (in preparation).

- Johnson, J. A., J. R. Hayes, D. D. Wagner, L. E. Carr and S. W. Joseph. 2001. Identification of Synercid® Resistance in Human *Enterococcus faecium* (in preparation).

- **Mechanisms of chemoprevention by dietary carotenoids and their metabolites in the prevention of chronic diseases in humans.** Frederick Khachik (UM), Eugene Mazzola, Shirley Blakely, and Andrija Kornhauser (FDA).

  Large quantities (30 g) of the following dietary carotenoids have been isolated from various natural products and have been purified by crystallization and chromatography: α-carotene, β-carotene, lycopene, ξ-carotene, photofluene, phytoene, lutein, and zeaxanthin. Corresponding quantities of the carotenoid metabolites 3-hydroxy-β,ε-caroten-3'-one, ε,ε-carotene-3,3'-dione, and 2,6-cyclolycopene-1,5-diol were prepared by partial or total synthesis.

  a) GAP-JUNCTIONAL COMMUNICATION ASSAY

  This chemopreventive mechanism of action for carotenoids and their metabolites has been investigated in collaboration with Professor John Bertram at the Cancer Research Center of Hawaii.

  1. Activity of carotenoids and their oxidation products as cancer chemopreventive agents in 10T1/2 cells. One possible explanation for the failure of supplemental beta-carotene to confluence cancer rates in the study of U.S. physicians is that the epidemiological association between lower risk and carotenoid consumption is based on the consumption of foods containing mixed carotenoids in contrast to highly purified and stabilized beta-carotene. We are in the process of evaluating the ability of a mixture of dietary carotenoids, designed to be similar in composition and molar ratio to that found in a "healthy diet", to inhibit the induction of carcinogen-induced neoplastic transformation in 10T1/2 cells. This was the same system in which we had shown single carotenoids have the ability to inhibit neoplastic transformation. The results so far have been confusing; over the dose range of 10^{-5}-10^{-6}M, concentrations previously shown with single agents to be active, the incidence of transformed foci was increased. This increase was approximately 4-fold (from 6 transformed foci in carcinogen-only treated controls, to 24 foci in cultures also treated with carotenoids) at the concentration of 10^{-5}M. These experiments are currently being repeated and extended to an examination of the influence of this carotenoid mixture on connexin 43 expression. Expression of this gene has previously served as an excellent marker of chemopreventive efficacy after treatment with both retinoids and carotenoids. If these results are indeed repeatable, under conditions where single carotenoids again show effectiveness, the studies have the potential to shed light on the very worrying evidence from two beta-carotene intervention trials conducted in smokers, that supplemental beta-carotene increases lung cancer risk.

  2. Activity of carotenoids oxidation products as modifiers of

differentiation in human keratinocytes. Studies have so far focused on the ability of lycopene, and its oxidized derivative 2,6-cyclolycopene-1,5-diol, found in the human diet and in plasma, to modify markers of differentiation in HaCaT cells. In addition, we have assessed the feasibility of using suspension cultures of HaCaT cells to induce differentiation in order to avoid the difficulties and longtime delays in assays where HaCaT cells are cultured as organotypic monolayers at the air/liquid interface. We believe we have succeeded in this venture, and now can induce terminal differentiation in these cells over a three-day period as opposed to the 14-day interval required for organotypic cultures. Using this new technique, which involves suspension in soft data, we can achieve reproducible increases in the expression of the mature keratin K10, with little change in the basal keratin K5. Expression of connexin 43 is also induced as would be expected of terminally differentiating cultures. Using this system, we have shown that retinoic acid will reduce expression of K10, as it does in organotypic culture and in intact skin, thus validating this model. Exposure of these cultures to highly purified lycopene and its oxidation product, cyclolycopene, also causes decrease in K10 expression, notably however, cyclolycopene exerts substantially greater effect then the parent molecule. Thus our predictions that oxidized products of carotenoids have greater biological activity have been borne out by these studies.

In contrast to be profound changes in expression of K10, expression of loricrin, a gene considered to be expressed later in the process of differentiation than K10, was little influenced by treatment with retinoic acid or lycopene. We do not fully understand the reasons for these different responses between the two genes. Since loricrin was shown to be expressed in these differentiating cells this does not reflect the failure to terminally differentiate. Because of difficulties in the supply of this commercial antibody (there is only a single source) we have only been able to perform this experiment once.

3. Determination of the molecular mechanism of action of selected carotenoid oxidation products. Our studies to date have focused on the use of reporter genes to probe the molecular action of retinoids. Because of the ease-of-use, dynamic range and availability of a commercial system that allows internal normalization to correct for differences in transfection efficiency, we have developed the dual Luciferase assay system for use in carotenoid research. In this system the promoter region of the gene of interest is positioned upstream of a fire-fly Luciferase gene which is then transfected into recipient cells. Activation of expression of this gene can be monitored by addition of the appropriate substrate and measurement of light output in a Luminometer. For purposes of comparison between cultures it is important to normalize for differences in transfection efficiency, in the dual Luciferase assay system this is achieved by co-transfection of a jellyfish-derived gene under the control of a constitutive promoter. Expression of this gene can also

be measured by light output from a different substrate. Changes in expression of the promoter of interest are then expressed as a ratio of the two emissions. We have so far focused on the role of carotenoids in suppressing transcription from AP1 responsive genes. The rationale behind this approach is that genes whose activity is modified by carotenoids, K10, loricrin and connexin 43 all contain AP1 sites in their promoter regions. Furthermore, the ability of retinoids to suppress activity of the first two genes appears to be a consequence of interference with AP1 signaling, while the tumor promoter TPA which antagonizes the biological effects of both retinoids and carotenoids, is known to increase AP1 signaling by virtue of its effects on protein kinase C.

b) PHASE 2 (DETOXICATION) ENZYMES ASSAY

Detoxication (phase 2) enzymes including quinone reductase [QR; NAD(P)H: (quinone acceptor) oxidoreductase, EC 1.6.99.2] are transcriptionally induced in many mammalian cells by low concentrations of a wide variety of chemical agents and such induction is associated with reduced susceptibility to chemical carcinogenesis. Eight chemical classes are currently recognized as inducers. In collaboration with Dr. Paul Talalay and Jed Fahey (Brassica Chemoprotection Lab., Johns Hopkins University), we have shown that carotenoids and their metabolites represent a new class of inducers and that some of these compounds are very potent. Bioassay of the induction of quinone reductase (QR) activity demonstrates that there is high inducer activity from certain of these carotenoids. Carotenoids therefore appear to have considerable ability to induce Phase 2 detoxication enzymes, and may have potential significance as means of detoxifying xenobiotics and acting as chemopreventive agents.

Purified carotenoids were dissolved in tetrahydrofuran (THF) and their quinone reductase (QR) inducer potency was determined by a coupled tetrazolium dye assay performed on digitonin extracts of Hepa 1c1c7 cells grown in microliter plates. Protein content of digitonin extracts was determined using a bicinchoninic acid assay. Carotenoids were tested over a concentration range of 0.78 μM to 100 μM and the final concentration of THF was 0.5% by volume. Activity is reported as follows: Concentration for Doubling (CD) inducer activity is the amount of compound required to double the QR activity of a microtiter plate well, initially seeded with 10,000 Hepa 1c1c7 (murine hepatoma) cells and containing 0.15 ml of a-MEM culture medium amended with 10% fetal calf serum, streptomycin and penicillin.

It is interesting to note that an acyclic carotenoid, lycopene, which lacks any functional group appears to be a reasonably good inducer, suggesting that the conjugated polyene chain itself has inducer ability. If one considers α-carotene (CD = 100 μM) with 10 conjugated and one isolated double bond to be the "parent" carotenoid, then by simply altering the position of one double

bond in the end-group, the phase 2 enzyme inducer potency in β-carotene (CD = 14 μM) with 11 double bonds is increased by 7-fold. This, of course, should be considered in the absence of any other structural changes.

Carotenoids therefore appear to have considerable potential to induce Phase 2 detoxication enzymes, and may have potential significance as means of detoxifying xenobiotics. The relevance of these findings is bolstered by the fact that serum and tissue concentrations of carotenoids are in the micromolar range and are therefore comparable to those required to produce Phase 2 enzyme induction.

Publication status:

1) Khachik, F.; Cohen, L.; Zhao, Z. “Metabolism of Dietary Carotenoids and Their Possible Role in Prevention of Cancer and Macular Degeneration” In: Functional Foods for Disease Prevention I, Shibamoto, T., Terao, J., Osawa, T. (Eds.), American Chemical Society Symposium Series, Oxford University Press, Chapter 7, page 71-85, 1999.

2) Khachik, F.; Bertram, J.S.; Huang, M.T.; Fahey, J.W.; Talalay, P. “Dietary Carotenids and Their Metabolites as Potentially Useful Chemopreventive Agents Against Cancer.” In: Antioxidant Food Supplements in Human Health, Packer, L.; Hiramatsu, M.; and Yoshikawa, T. (eds), Academic Press, Tokyo, Chapter 14, page 203-229, 1999.

3) Khachik, F.; Steck, A.; Pfander, H. “Isolation and Structural Elucidation of (13Z,13’Z,3R,3’R,6’R)-Lutein from Marigold Flowers, Kale, and Human Plasma” J. Agric. Food Chem., 47: 455-461, 1999.

4) Cohen, L. A.; Zhao, Z.; Pittman, B.; Khachik, F. “Effect of Lycopene on N-Methylnitrosourea-Induced Mammary Tumorigenesis” Nutrition & Cancer, 34: 153-159, 1999.

5) Bertram J.S.; King, T.; Fukishima, L.; Khachik, F. “Enhanced Activity of an Oxidation Product of Lycopene Found in Tomato Products and Human Serum Relevant to Cancer Prevention” In: Antioxidant and Redox Regulation of Genes, Sen, C.K.; Sies, H.; Baeuerle, P.A. (eds), Academic Press, San Diego, Chapter 18, page 409-424, 2000.

6) Scholz, T.A.; Hata, T.R.; Pershing, L.K.; Gellermann, W.; McClane, R.; Alexeeva, M.; Irmakov, I.; Khachik, F. “Non-Invasive Raman Spectroscopic Detection of Carotenoids in Human Skin” J. Invest. Dermatology, 115 (3): 441-448, 2000.

7) Bernstein, P.S.; Khachik, F.; Carvalho, L.S.; Muir, G.J.; Zhao, D.Y.; Katz, N.B. "Identification and Quantitation of Carotenoids and Their Oxidation Products in the Human Eye" Submitted to Exper. Eye Res., July 2000.

8) Sweeney, C.T.; Helzlsouer, K.J.; Khachik, F. "The Association Between Selected Carotenoids and Their Metabolites and the Risk of Developing Lung Cancer" Submited to Cancer Epidemiology, Biomarkers, and Prevention, May 2000.

Abstracts and invited presentations:

1) F.Khachik "Development of a Multicarotenoid Dietary Supplement for Chemoprevention of Cancer and Macular Degeneration" 2nd International Conference on Food Factors, Chemistry and Health Promotion, Kyoto, Japan, December 12-17, 1999.

2) F.Khachik "Distribution, Bioavailability, and Metabolism of Lycopene in Humans" Prostate Cancer Prevention 2000: The Role of Nutrition, Organized by Faculty of Medicine, University of Toronto, Toronto, Canada, March 3-4, 2000.

3) Khachik, F. "Distribution of Carotenoids in Human Serum and Tisssues" First South East Asia and Pacific Regional Meeting on Carotenoids, Bangkok, Thailand, August 2-5, 2000.

4) Khachik, F. "Update on Carotenoid Analysis" First South East Asia and Pacific Regional Meeting on Carotenoids, Bangkok, Thailand, August 2-5, 2000.

5) Humphries, J.; Graham, R.; McIntosh, G.; Worsley, T.; Khachik, F. "The Role of Carotenoids in Human Health" First South East Asia and Pacific Regional Meeting on Carotenoids, Bangkok, Thailand, August 2-5, 2000.

6) Khachik, F. "Bioavailability and Metabolism of Dietary Carotenoids" International Conference and Exhibition on Nutraceuticals and Functional Foods, Houston, Texas, USA, Sept. 14-17, 2000.

7) Khachik, F. "Mechanistic Studies on Carotenoids and Their Metabolites in the Prevention of Chronic Diseases" International Conference and Exhibition on Nutraceuticals and Functional Foods, Houston, Texas, USA, Sept. 14-17, 2000.

Second year projects:

- **Immunologic Sequela Following Oral Exposure to a Foodborne Toxin.** Carol Pontzer (UM), Richard Raybourne and MaryAnn Principato (FDA)

  This project addresses the degree of association between the levels of oral exposure to staphylococcal enterotoxins (SE) and increased risk for development/exacerbation of immune-related diseases. Last year, the first demonstration of the dose-related effect of oral SE on compartmentalization of immune responses was reported. At low oral toxin concentrations only the gut-associated lymphoid tissue is affected, but at high concentrations, SE can gain access to the systemic immune system, precipitating dramatic immunological changes. The importance of systemic T cell activation is that it is responsible for both the relapse of quiescent autoimmune disease and further T cell depletion in AIDS patients. We have now extended our initial observations of increased apoptosis of systemic T cells upon high-dose oral toxin exposure. Mice were given oral SEB at either low (50-140 mg) or high (400 mg) doses, and the extent of apoptosis was assessed in situ in spleen, PeyerÕs patches and mesenteric lymph nodes by TUNEL assay. Two days following exposure to all concentrations of oral toxin examined, significant apoptosis was observed locally in PeyerÕs patches and mesenteric lymph nodes as well as systemically in spleen. While locally exposed T cell death is consistent with previous observations of T cell activation and changes in cell cycle, systemic elimination of T cells with low-dose toxin exposure was unanticipated. This indicates that the threshold for adverse systemic immune effects may be appreciably lower than those producing emetic or toxic shock responses.

  Of interest, while apoptosis was observed in the parenchymal or T cell area, dose dependent disruption of the architecture of the follicles or B cells areas was also observed. This may be due to toxin-stimulated B cell proliferation. By day 6 after oral SEB exposure, tissues from mice receiving low dose toxin had apoptotic cells in B and T cell areas of all three tissues. Consistent with the apoptosis evident at day 2, mice examined six days following high-dose SE exhibited significantly reduced cellularity of both local and systemic lymphoid organs. In effect, the tissues had been effectively cleared of cells. Thus, while the high-dose toxin causes extensive lymphocyte death, lower exposure levels also induced systemic immune abnormalities. Further, the B cell compartment was affected as well as CD4+ T cells. Alterations of B cells by SE has been reported in vitro, but this is the first demonstration of in vivo effects of oral SE on B cells. In light of B cell alterations, antibody responses to oral toxins will be assessed.

  Differences had also been observed in the splenic CD4+/CD8+ T cell

ratio between young and old mice. In order to confirm this observation, the local and systemic in situ effects of exposure to high dose oral SEB was determined in aged mice (1.5-2 years of age). In general, the time frame during which cell death could be observed was attenuated in the older animals, i.e. apoptosis of splenic and PeyerÕs patch T cells was delayed until at least day 4 in aged animals. Since gut associated CD4+ T cells are associated with production of disease, an increase in SE-induced emetic disease severity would be predicted in aged individuals. Further, splenic T cell apoptosis was reduced relative to the young animals. Thus, these cells would remain activated for extended periods of time and potentially increase the development of autoimmune disease subsequent to foodborne SE.

An additional goal is determination of the utility of a toxin synthetic peptide as a vaccine candidate to prevent toxin transcytosis across the gut epithelium and ameliorate systemic immune dysfunction. In year one, the transcytosis system had been established and a candidate peptide incorporating a 10 amino acid sequence KKKVTAQELD had been identified. The dose-dependent movement of SEB across gut epithelial monolayers has been confirmed in the Caco-2 system. Caco-2 cells are a human adenocarcinoma line that forms epithelial monolayers. The electrical resistance of these monolayers is reduced relative to the crypt-like colonic epithelial T-84 cells used for drug absorbtion modeling, so that more toxin moves across the monolayers. Nontheless, dose-dependency was observed. The transcytosis of other SE has also been examined. While movement of SEA across gut epithelial monolayers was equivalent to that of SEB, 3-fold more TSST-1 was transcytosed at the lower doses, indicating a greater risk for systemic disease from low level TSST-1 exposure. The toxin synthetic peptide that has been expressed for in vivo vaccine trials has been used to determine its effect on toxin transcytosis. At a peptide:toxin ratio of 10:1, movement of SEB across the gut monolayers was reduced by over 90%. This is in contrast to a lack of effect on toxin transcytosis by a control toxin peptide of the same molecular weight but corresponding to different region of the molecule that has previously been shown to affect T cell function. Thus, it has been established that the sequence originally identified is indeed important for movement of SE from the gut into the body. Further, the domain of the toxin molecule that is involved in toxin transcytosis is distinct from that eliciting T cell activation. Finally, this transcytosis sequence is one of two highly conserved sequences among the SE. This reinforces the concept of a single vaccine capable of blocking transcytosis of multiple toxins and preventing systemic immune disease.

Publication status

Pontzer, C.H., Karmazyn, Y., Raybourne, R., and Principato, M.A. 2000.

Oral Staphylococcal Enterotoxin Exposure Elicits Local and Systemic Lymphocyte Apoptosis. (manuscript in preparation).

- **The Missing Connection: Isolation and concentration of Microorganisms on Biocapture Surfaces.** Jonathan Bundy, Catherine Fenselau (UM), Mary Carson and David Wagner (FDA)

Work in the final year of this project focused on further refining and evaluating the carbohydrate biocapture surfaces developed in the previous year, applying their use to contaminated food samples.

In order to determine the optimal carbohydrate for binding the microbial targets, four microbial samples (*E.coli* O157:H7, *E. coli* K-12, *S. typhimurium*, and *C. jejuni*) were challenged separately with five different carbohydrate surfaces constructed in the laboratory bearing either glucose, mannose or one of the Lewis carbohydrate antigens (a, b, or x). These samples were then analyzed by mass spectrometry, and the degree of binding to the surface was evaluated qualitatively by the number of peaks observed and the signal strength of the resulting mass spectrum. *E.coli* O157:H7 bound well to all of the carbohydrate targets, in contrast, *C. jejuni* only showed significant binding by mass spectrometry when exposed to a Lewis-x surface. The other two species exhibited weak binding to some carbohydrate targets and stronger binding to others.

The lectin and carbohydrate surfaces were then challenged with microbial samples spiked into food matrices such as milk, chicken package exudate, chicken carcass rinsing and urine. Microorganisms were detectable from all of these matrices using the carbohydrate surfaces, which in general, showed superior results when compared to the lectin surfaces. In urine samples, an order of magnitude increased sensitivity was observed from carbohydrate surfaces, as compared to lectin surfaces. This may be due to the higher density of ligands on the carbohydrate polymer used to construct these surfaces. In addition, the high intrinsic carbohydrate content of some of these matrices was expected to cause difficulties with the use of the lectin surfaces in some situations. This was illustrated in an experiment where *S. typhimurium*-spiked milk was exposed to both a Concanavalin A lectin surface and a Lewis-b carbohydrate surface. No binding of the microorganisms was detectable using the lectin surface, presumably due to the blocking of the lectin binding sites by carbohydrate moieties in the milk sample. In contrast, the carbohydrate-bearing surface allowed *S. typhimurium* to be detected. Similar results were also observed with chicken package exudate. Intrinsic bacterial contamination was also observed in mass spectra of the unspiked exudate sample, although it could not be identified conclusively by mass spectrometric means. Whole egg and egg white samples spiked with microorganisms were also evaluated with both types of surfaces but no binding of microorganisms

could be detected, possibly due to the high protein content of these matrices.

Publications and Presentations

1. Bundy, J. L.; Fenselau, C. Lectin and Carbohydrate Affinity Capture Surfaces for Mass Spectrometric Analysis of Microorganisms. *Anal. Chem.* 2000, in press.

2. Bundy J.L. Development of Biocapture Surfaces for Mass Spectrometric Analysis of Microorganisms. Seminar Presented to the Center for Veterinary Medicine, Food and Drug Administration, October, 2000.

3. Bundy J.L. Development of Biocapture Surfaces For Mass Spectrometric Analysis of Microorganisms. Seminar Presented to the Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, September, 2000.

4. Bundy, J. L.; Fenselau, C. Carbohydrate Affinity Capture Surfaces for Mass Spectrometric Analysis of Microorganisms. Presented at the 48th ASMS Conference on Mass Spectrometry and Allied Topics, Long Beach CA, June, 2000.

- **The Detection of Foodborne Pathogens in Biofilms Using Antibodies, Lectins, and Fluorescent Dyes.** Ronald Weiner, Sam Joseph, Lewis Carr (UM) and Dr. Ben Tall (FDA)

  Foodborne pathogenic microorganisms can form biofilms in which they elude detection and resist removal. It was found that Calcofluor, which binds to the complex polysaccharides that comprise a large portion of the biofilm matrix, fluoresces to indicate potential biofilm contamination. It bound to *Salmonella berta, S. typhimurium, Aeromonas hydrophilia,* and *Campylobacter jejuni*, in other words, all pathogens that were tested. The sensitivity of this inexpensive reagent was determined with respect to its optimum concentration and the extent of biofilm contamination. In a potentially exciting development, it was found that Calcofluor can be used *in situ* in food processing plants to identify contamination. This test would be easy to do and relatively inexpensive.

  Lectins were also identified that were specific probes of specific foodborne pathogens. However, unlike the case for Calcofluor, it was found that the lectins were not unambiguously selective enough for routine field use, especially considering their relatively high cost.

Additionally, conditions were examined under which *Salmonella typhimurium* strain DT 104, perhaps the most important and prevalent food pathogen, was likely to produce removal-resistant biofilms. An exciting finding of this investigation was that, in this strain, biofilm accretion was temperature, growth medium, and oxygen dependent. The exopolysaccharide capsule was purified and antibodies against it were prepared for use as a diagnostic probe and to study environmental factors that increase the virulence of *Salmonella typhimurium* strain DT104.

Publication status:

1. De Renzende, C., E. Teicher, L. Carr, B. Tall, R. Weiner and S. Joseph. 2000. Detection of food-borne pathogens using Calcofluor and lectins. SIM International Symposium on Food-borne Pathogens. Crystal City, VA (abstract).

2. Sledjeski, D. and R. Weiner. 2000. Regulation of EPS synthesis by nutrient concentration in *Shewanella colwelliana*. Appl. Environ. Microbiol. (In manuscript).

3. De Renzende, C., E. Teicher, L. Carr, B. Tall, R. Weiner and S. Joseph. 2000. Detection of food-borne pathogens using Calcofluor and lectins, J. Industrial Microbiol. Biotechnology (In manuscript).

4. Langille, S., G. Geesey and R. Weiner. 2000. Inhibition of *Hyphomonas rosenbergii* VP-6 capsular mediated adhesion by binding agents including metals, lectins and dyes. JIMB (In press).

5. Quintero, E., S. Langille and R. Weiner, 2000. Exopolysaccharide capsules of marine, prothescate bacteria specifically bind complexed and colloidal gold. JIMB (In review).

6. Weiner, R. M., M. Melick, K. O'Neill and E. Quintero. 2000. *Hyphomonas adhaerens* sp. nov., *Hyphomonas johnsonii* sp. nov., and *Hyphomonas rosenbergii* sp. nov., Marine Budding and prothescate bacteria. Int. J. Syst. Evolut. Microbiol. 50:459-469.

<u>First year projects:</u>

- **Viral Immunosuppression and the Infection of Shell eggs by *Salmonella enteritidis*.** Robert Heckert, Wenxia Song (UM) and Richard Raybourne (FDA)

  In the last 20 years there has been an increase in human food-poisoning outbreaks attributable to *Salmonella enteritidis* (SE) in the United States. Epidemiological studies of this increase have indicated that grade A shell

eggs are an important source of SE. Despite the tremendous efforts made by the poultry industry, no effective measures for elimination of SE colonization have been developed. In addition, the process by which laying hens become infected with SE and subsequently produce contaminated eggs remains unclear.

It is well known that the immune system plays an important role in all phases of a microorganism's infectious cycle, from invasion to clearance. The effect of the immune system on the degree of SE egg contamination has not been well studied. The influence of immunity on the pathogenesis of salmonellae in the laying chicken is unclear. In the poultry industry, it is well recognized that there are several immunosuppressive viruses present. This proposal seeks to investigate how immunosuppressive viruses affect the immune system and what role this may play in SE colonization, tissue invasion, persistence and bacterial contamination of the egg. The implied outcome of this work is that control or elimination of immunosuppressive viruses would also significantly limit SE infections, thereby leading to better poultry health, a decreased incidence of contaminated shell eggs and a reduction of exposure to consumers.

Goal A. Examine the effect of viral infection on SE colonization and the immune response in young birds

Specific Aim 1: Establish colonization and immunosuppression.

A method of establishing an SE infection in chicks was clearly developed using a specific strain of SE containing the green fluorescent protein (GFP). In addition it was shown by histology that these birds can be super infected with chicken anemia virus (CAV).

Specific Aim 2: Investigation of SE load and tissue invasion:

It has been shown that SE is secreted in large amounts early in the infection cycle and then persists at lower amounts for at least 8 weeks. By following the bacteria in the tissues by use of the GFP, it has also been shown that SE invades and persists in many tissues of the young laying hen. The bacteria were shown to first colonize in the lumen of the gut and then move into the rest of the tissues of the bird. In the time period examined thus far, this did not appear to be accentuated by the presence of CAV.

Specific Aim 3: Investigation of the immune status:

Most of the birds seroconverted to Salmonella with some having a very high antibody response. All of the birds also had a local IgA antibody

response in the intestine, however this was greatly diminished in the birds infected with CAV.

Specific Aim 4: Investigate the extent of the virus infection:

The CAV infection was shown by histology to cause severe thymus cell depletion, as expected. Several of the birds also showed severe depletion of red blood cells due to the viral infection in the progenitor cells of the bone marrow. These are all classical signs of CAV infection and indicate that we did induce a viral infection in these birds, which resulted in immune system disruption.

Goal B. Examine the effect of viral infection on SE long-term colonization, invasion shedding and the immune response in egg laying hens

This phase of the study has not yet been completed and therefore the long-term effects of CAV infection on Salmonella infection cannot be assessed.

Summary:

To date it has been possible to successfully and reproducibly establish a Salmonella-CAV super infection in young laying chickens. It has been established that Salmonella invades many tissues in the bird and is excreted in the feces for at least 8 weeks. During the time period evaluated, the super infection with CAV has not been shown to have any effect on the level of Salmonella infection or excretion. Studies are underway to repeat this procedure and examine the long-term effects of CAV infection on the Salmonella status of laying hens as they go into production.

- **Using a Probabilistic Risk Assessment Model to Study Risk of *E. coli* Contamination in Hard Cheeses.** Mohammad Modarres (UM) and Joseph Schlesser (NCFST, FDA)

  The objective of this research is to develop a probabilistic risk assessment model to study risk of *E. coli* contamination in rennet-coagulated cheese and particularly hard cheese (Cheddar). The objective is also to support regulatory decision-making and to establish a formal and systematic way to define the needs for additional research.

  In this research a number of probabilistic model-based tools and techniques developed and used in various engineering disciplines are being adapted for applications to food safety, and particularly food borne pathogens in cheeses. In this approach, food risk assessment involves the steps of screening to determine whether risks from a particular microbial

food-borne hazard reach a threshold of concern; estimating frequency of occurrence of particular hazards and their magnitude occurring at a specific location or at all locations (public risk) along the farm-to-fork path; determining risk-significant contributors to develop control mechanisms and define needs for more data; and providing the results of the risk assessment to regulatory decision makers.

Particularly, the probabilistic model-based tools being developed are being applied to evaluate the risk of *E. coli* O157:H7 in the cheese-making process, which allows determination of risk significant activities or events, best control strategies, areas where more data improve risk estimates and reduce uncertainties, and expected societal impacts due to exposure to this particular hazard.

Phase I effort is being focused on the analysis of the risk at the production level in the process of manufacturing of cheeses. The next phases expand to include “distribution” and “consumption”. Also, the cheese risk assessment application provides an example from which the general “model based” approach for food safety will be derived.

Research activities accomplished:

The literature on *E. coli* contamination of cheeses has been gathered and reviewed. A substantial amount of data has been gathered. This has led to the next step of developing a model to represent the cheese production process.

A risk-modeling concept called “Master Logic Diagram” (MLD) is adapted to the general cheese-making process. Due to the diversity of cheeses and the different cheese-making processes involved, the MLD has been limited to rennet-coagulated cheeses. In this MLD model, the manufacturing process had been divided into successively more abstract steps, and each step has been modeled taking into account the most critical variables, such as temperature and time affecting the growth of *E. coli*.

A generic model has been developed for rennet-coagulated cheeses, and only some representative types of cheeses such as cheddar, brick, cottage, Emmental, Camembert and Tilsit may be applied. However, the model has been developed in such a way that it can be easily adapted for use in modeling a variety of other cheeses.

Ongoing activities:

The database for assessing the statistical growth behavior of *E. coli* for important steps described in the rennet-coagulated cheese making process is being completed. This preliminary database has been oriented almost exclusively to the strain O157:H7; however, due to the limited available data for this specific strain, some generic data of *E. coli* and from other strains of *E. coli* (e.g. B2C, 1624, 4608) are being used.

The validation and adaptation of generic data constitutes the next stages of the research. Elicitation techniques using expert opinions and use of the Bayesian approach to combine expert opinion with actual data are being studied.

Identification of steps into the cheddar cheese-making process where *E. coli* grows or decreases significantly is being carried out. This identification involves determining conditions and parameters that affects growth of *E.* coli. Up to now, some risk (contamination) multiplicative factors have been determined for some specific steps and conditions; however, when more data are obtained a well-defined probabilistic distribution can be developed.

Master Logic Diagram (MLD) for the general cheddar cheese-making process will be developed from the generic model currently being developed. In a similar way to the general cheese making process, the manufacturing process has been decomposed into its more important steps, and each step has been modeled separately, taking into account the most critical variables. These models are being integrated. Particularly, in this task we are working on modeling the ripening or maturing of cheddar cheese as a stochastic process, either using an empirical model, or if possible, using a physically-based model.

Expected products:

Development of model is on schedule and completion of the model, its computer implementation and some risk analysis runs are anticipated by December 20, 2000. A final report for phase I of this research will be completed by the end of December 2000. One paper has been submitted for presentation at the next Society for Risk Analysis Conference in December 2000.

- **Antibiotic Resistance Integrons in Shiga Toxin-producing *Escherichia coli* and *Campylobacter jejuni/coli*.** Jianhong Meng (UM), David White, S. Zhao, and David Wagner (FDA)

The following three studies have been conducted during the first year of the project.

1. Identification and Characterization of Integron Mediated Antibiotic Resistance among Shiga Toxin-Producing *Escherichia coli*

Fifty-seven isolates of Shiga toxin-producing *Escherichia coli* (STEC), including 29 *E. coli* O157:H7 and 28 non-O157 STEC, were analyzed for antimicrobial susceptibilities and the presence of class 1 integrons. Sixty-eight (n = 39) percent of isolates exhibited resistance to two or more antimicrobial classes. Multiple resistance to streptomycin, sulfamethoxazole, and tetracycline was most often observed. Class 1 integrons were identified among 9 STEC isolates including serotypes O157:H7, O111:H11, O111:H8, O111:NM, O103:H2, O45:H2, O26:H11, and O5:NM. The majority of amplified integron fragments were 1 kb in size with the exception of one *E. coli* O111:H8 isolate which possessed a 2 kb amplicon. DNA sequence analysis revealed that the integrons identified within the O111:H11, O111:NM, O45:H2, and O26:H11 isolates contained *aadA* gene encoding resistance to streptomycin/spectinomycin. Integrons identified among the O157:H7 and O103:H2 isolates also possessed a similar *aadA* gene. However, DNA sequencing revealed only 86% and 88% homology, respectively. The 2-kb integron of the *E. coli* O111:H8 isolate contained three genes, *dhfr*XII, *aadA*2 and a gene of unknown function, *orfF*, which were 86%, 100%, and 100%, homologous to previously reported gene cassettes identified in integrons found in *Citrobacter freundii* and *Klebsiella pneumoniae*, respectively. Furthermore, integrons identified among the O157:H7 and O111:NM strains were transferable via conjugation to another strain of *E. coli* O157:H7 and to several strains of *Hafnia alvei*. These results indicate that STEC have developed multiple antimicrobial resistance phenotypes which can be partially attributed to the acquisition of integron mediated gene cassettes.

2. Genomic DNA Fingerprinting of *Campylobacter* Isolated from Retail Poultry Meats by Ribotyping and Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) and ribotyping are useful tools for subtyping bacterial pathogens. A total of 115 *Campylobacter* (71 *Campylobacter jejuni* and 44 *Campylobacter coli*) isolated from 47 poultry meat samples from retail stores in Maryland Suburban were analyzed by PFGE with *Sma*I and ribotyping with *Pst*I. PFGE and ribotyping both gave distinct groups of the organism at the species level. They also were able to differentiate isolates under species. The 71 *C. jejuni* were grouped into 32 PFGE patterns and 19 RiboGroups, whereas

44 *C. coli* to 18 PFGE patterns and 7 RiboGroups. A total of 50 PFGE patterns and 26 RiboGroups were identified among the 115 *Campylobacter* isolates. The combination of RiboGroups and PFGE patterns gave 68 genotypes, of which 54 had multiple PFGE patterns within one RiboGroup, and 31 had multiple RiboGroups within one PFGE pattern. Several genotypes were observed in isolates obtained from different supermarket chains at different sampling times. The results showed that PFGE had a higher discriminatory power than ribotyping, and the two methods were complementary in typing *Campylobacter.*

3. Evaluation of E-test and Standard Agar Dilution for Antibiotic Susceptibility Testing of Campylobacter

The objective of this study was to evaluate the methods of Etest and agar dilution on the antibiotic susceptibility testing of 135 *Campylobacter jejuni* and *Campylobacter coli* retail meat isolates against 8 antibiotics. The agreement between these two methods ranged from 32.6% for nalidixic acid, 51.9% for chloramphenicol to 92.6% for gentamicin. Agar dilution, a method recommended by NCCLS, may be a good choice for testing a lot of isolates. The biggest challenge for agar dilution was the swarming growth of the inocula. Etest is quite flexible when a few isolates are tested against several drugs. Swabbing for inoculation can be labor intensive, when dealing with lots of isolates. In addition, interpretation of results is quite subjective. The biggest challenge of Etest was the poor growth of the isolates on the test agar.

- **The Evaluation and Removal of Bacterial Biofilms from Food and Food Processing Materials.** Paul Schreuders (UM) and L. Ali (FDA)

It is known that bacteria can attach to materials that are used in the food processing industry. Over time, biofilms can form. These films are more robust to chemical and mechanical treatment than free-floating cells. The research problem involves the development of a method for examination of biofilms on materials frequently used in the food processing industry. The biofilms of interest consist of the pathogen strain *E. coli* O157:H7. Effort is made to investigate if the strain grows different rates on different substrate materials, how and if the shape of the biofilm spots changes over time, and what methods of surface treatment are efficient in the reduction of the biofilm.

Culture and Analysis Methodology Development

Development of methods for the culture and analysis of bacterial biofilms on food processing materials has been completed. Initially, this work was performed on the *E. coli* strain K12, a harmless strain of *E. coli*. This study investigated biofilms grown on three different materials: glass,

polished 316 stainless steel, and brushed 316 stainless steel. Both materials are commonly used in the preparation and display of food. The examination investigated the relationships between substrate material and biofilms. Two classes of biofilm properties were examined; overall growth kinetics and colony morphology. A model system has been developed in which the bacteria and the biofilm can be examined while still present on the chosen material. The bacteria cultured using this *in situ* technique were stained to evaluate their viability and morphology using various fluorescent probes and epifluorescence microscopy.

During the development of the methodologies, the effects of ozone and deionized water on biofilms grown on the substrates were compared. The values of percentage area, absolute area, perimeter and circularity of the biofilm colonies were analyzed using the software package SAS. No difference in growth over material and time was found when analyzing the percentages of coverage of biofilms for slides that had been treated with ozone or DI water. In the case of the absolute area, for slides rinsed in deionized water, differences were found depending on what material the biofilm was grown on, and there was also statistical differences over time. Further, the perimeter was only affected by time, and the circularity was affected by material. The shapes of the biofilms grown on brushed steel have the greatest circularity, hence, the most irregular shape.

Preliminary Results Using *E. coli* O157:H7

Once a method for the growth of biofilms in lab scale and a general staining protocol was developed, the work and investigation of *E. coli* O157:H7 was initiated. The strain was grown on Petri plates over night, and transferred to TSBYE1/3X medium (tryptic soy broth, 30 g/liter plus yeast extract, the mixture is diluted three times) for growth over night. Once the medium and bacteria had reached the optical density of 0.7, the tank was inoculated. The ratio of dilution of inoculating medium to medium in the tank was 1:1500. Sampling was performed 24, 51, 72 and 145 hours after the tank was started. When sampling, the slides were rinsed in deionized (DI) water for 5 minutes, prior to staining. Two tanks have been run using only DI water as treating agent. On one occasion, sampling was also performed 6 and 20 hours after the tank was started, and biofilms had already been developed to certain extent on polished and brushed stainless steel. One tank has been run using ultrasound as treating agent. The results are yet to be analyzed, but there is a large visual difference in amount of biofilm left on ultrasound-treated slides compared to DI-treated slides.

Color Recognition and Analysis Software

A program is under development for automatic analysis of stained samples using color recognition. The program will be tested and modified based on the ability of the testing to reach a satisfactory reliability. The program will then be complete and can be used for automatic information collecting and examination of stained samples. Since the color recognition program will not specify the color of the stains, it will be possible to use it for any other color-stained studies.

Publication status:

1. Schreuders, P.D., Lomander, A., and Rinko, L.J., "The Implications of Biofilm Formation: Manipulation and Control Strategies," in Biological and Biotechnical Resources, Y.C. Tripathi, editor. (Book Chapter - In Submission)

2. Lomander A. Schreuders P.D., and Ali, L.H., "Analytical Techniques for the Evaluation of Biofilms on Food Processing Materials," Northeast Agricultural/Biological Engineering Conference, Ithaca, NY, August 1-4, (2000). (Abstract)

3. Andrea Lomander, A., Schreuders' P.D., Russek-Cohen, E, and Ali, L., "The Kinetics of Microbiological Contamination on Food Processing Materials" *(Manuscript in preparation for submission to the Journal of Food Protection)*

- **Communicating with Consumers about Dietary Supplements.** Laura Sims (UM), J. Guthrie, Alan Levy, and B. Derby (FDA).

  The project was initiated in September 2000 with arrival of a postdoctoral research associate. The grant was terminated in November 2000 following departure of Professor Laura Sims for a position at the University of North Carolina – Greensboro, departure of Dr. J. Guthrie from FDA for another position, and resignation of the postdoctoral research associate. Unused funds are being returned from the department to JIFSAN.

<u>Specific Research Initiatives:</u>

- **Mechanistic Assays for the Phototoxicity of Cosmetics.** Daniel Falvey and Peter Vath (UM), and Wayne Wamer (FDA)

  The long term objective of this research is development of non-animal assays that predict the risks associated with the use of cosmetic ingredients on sun-

exposed skin. We hypothesize that photophysical measurements, characterizing the formation and decay of a potential photosensitizer's excited states under biologically relevant conditions, and in vitro photobiological measurements, characterizing a potential photosensitizer's cytotoxicity and cellular targets, provide the mechanistic information needed for estimating acute and chronic phototoxic risks. We have carried out a series of photophysical and photobiological studies aimed at examining potential harmful effects of cosmetic ingredients that are commonly applied to sun-exposed skin. Work to date has focused on organic components of aloe vera gel and extracts: aloe emodin and rhein.

Mechanisms for the phototoxicity of aloe emodin have been studied using laser flash photolysis. Possible mechanisms include singlet oxygen formation and the production of reactive intermediates following irradiation with UV light. Aloe emodin was found to produce singlet oxygen with a quantum yield of 0.50 in acetonitrile.

Laser flash photolysis (LFP) experiments were carried out on aloe emodin in acetonitrile using 355 nm light as the excitation source. The transient spectrum has absorption bands due to the triplet state of aloe emodin at 480 nm and 690 nm. The energy of the aloe emodin triplet state was estimated to be between 42 and 49 kcal/mol from triplet quenching experiments. Repeating the LFP experiment in 40% acetonitrile/water results in a transient spectrum with a longer-lived absorption band at 520 nm in addition to the absorption bands from the triplet state of aloe emodin. The absorption band at 520 nm in the transient absorption spectrum corresponds almost exactly with the steady state absorption spectrum of aloe emodin in basic solution, and was therefore assigned to the aloe emodin anion. The absorption at 520 nm was not observed when the LFP experiments were carried out in 40% acetonitrile/water at pH = 3.7. Two pKa values were determined for aloe emodin from titration experiments. The first was at 3.2 and the second at 7.4. LFP experiments carried out on the related compound 1,8-dimethoxy-3-(hydroxymethyl)-9,10-anthraquinone give transient absorption spectra with absorption bands only due to the triplet state. This supports our previous observations since 1,8-dimethoxy-3-(hydroxymethyl)-9,10-anthraquinone cannot lose a proton to form the anion.

LFP experiments were also carried out on aloe emodin in the presence of the electron donor diphenylamine. The transient absorption spectrum has new absorption bands at 450 nm and 490 nm. These absorption bands have been assigned to the protonated semiquinone and the semiquinone anion, respectively. The proposed mechanism involves electron transfer from diphenylamine to the excited singlet state of aloe emodin to form the semiquinone anion, followed by protonation to form the protonated semiquinone.

The related compounds aloin and rhein have also been studied by LFP. A transient absorption band due to its triplet state was observed for rhein, while no transient absorption bands were observed for aloin. Rhein was also found to produce singlet oxygen with a quantum yield of 0.41 in acetonitrile. Finally, binding of aloe emodin to DNA was studied by fluorescence quenching, resulting in a binding constant of 1.2×10^4 mol^{-1}.

- **Developing Methodology to Detect Adverse Events.** Johnny Blair, Timothy Triplett, Henry Wu, and Song Zhao (UM), and Marilyn Flack (FDA)

  The goal of this research is to develop a model which will provide mechanisms to collect adverse event data which will provide access to risk management information to the primary users of this database (risk managers, epidemiologists, administrators, etc. - depending on the type of facility reporting the adverse event). The research is designed to analyze the components of a reporting system which the public users of data in the reporting system database will find most useful in conducting risk hazard and risk management analysis. As modules of the model are developed, they will be piloted with the user community. This research involves the Office of Academic Computer Service and the Suvey Research Center in the College of Behavioral and Social Sciences, University of Maryland. Although the research is using a medical device scenario as its model, the model once developed could be used by other groups in developing risk management policies.

  The supplemental funding for this project was awarded in mid-August. The project was initiated in mid-September. A prototype of the web-based Adverse Event Reporting System is anticipated to be available in November 2000.

Leveraging:

Development of Research Partnerships:

The development of partnerships with external constituencies is one of the major avenues JIFSAN uses to expand the science base available for addressing public health policy issues. UM and FDA scientists have begun collaborative research efforts with other organizations. These projects will contribute to the science for current and future regulatory issues and activities that impact on public health policies and are closely aligned with the FDA's research needs.

- Seed money that JIFSAN provided through two projects (previously described) to Dr. Wenxia Song and Dr. Robert Heckert during the past three years enabled them to generate the preliminary data necessary to successfully obtain a competitive USDA grant. This grant from the USDA’s National Food Safety Initiative is for $600,000 over a period of three years and involves Dr. Heckert as PI and co-PIs from the

University of Maryland (Dr. W. Song), USDA Agricultural Research Service (Dr. H. Lillihoj), and FDA (Drs. R. Raybourne and Uma Babu).

- **The Seychelles Child Development Study.**

Through JIFSAN, scientists at the University of Maryland are cooperating with colleagues from the University of Rochester in conducting a pilot study on human neurobehavioral outcomes of children who participated in the Seychelles Child Development Study "Years Following Prenatal and Postnatal Exposure to Methylmercury from a Fish Diet." JIFSAN played a critical role in bringing together the resources to continue this long-term study on the developmental effects of mercury. Funding for the project was provided by the FDA (through a supplement to the JIFSAN Cooperative Agreement), the Electric Power Research Institute ($486,000), the National Tuna Foundation ($10,000), and the National Fisheries Institute ($5,000).

The purpose of the study was to develop a battery of neurodevelopmental measures capable of detecting subtle CNS dysfunction in children. A battery of tests has been designed to explore functional disabilities in children arising from adverse conditions during early development. Although the battery was prompted originally by questions arising from methylmercury exposure, it was also designed for applicability to neurotoxicant exposures arising from pesticides, solvents, persistent organic pollutants, other metals, and nutrients. The test battery includes the following categories: (1) neuropsychological tests with established psychometric properties not widely exploited in studies of developmental neurotoxicity; (2) electrophysiological and behavioral tests of sensory functioning spanning a broader range of indices than those used generally in studies of neuropsychological development; and (3) adaptations of performance tasks used previously only in animals. The battery was developed in Rochester, New York, then field-tested on a group of 61 Seychellois children. Findings suggest a number of tests and procedures with the potential for inclusion in test batteries aimed at the exploration of developmental neurotoxicity.

Funding for this study was completed during the year included in this annual report. A final report on the project will be filed with JIFSAN in early 2001.

Publication status

Philip W. Davidson, Bernard Weiss, Gary J. Myers, Deborah A. Cory-Slechta, Becky J. Brockel, Edna Carter Young, Mark Orlando, David Loiselle, Donna Palumbo, Randal Pitelli, and Jean Sloane-Reeves. 2000. Evaluation of Techniques for Assessing Neurobehavioral Development in Children, NeuroToxicology, In press.

- **FT-NIR Rapid Determination of Food Integrity**

Food supplies are among the most vulnerable routes for the delivery of lethal or incapacitating quantities of chemical or biological agents. The goal of this project is to develop methodology for the rapid detection of contaminants (chemical and microbial) in a wide range of foods by using FT-NIR spectroscopy combined with multivariate data analysis techniques. The data obtained can lead to the development of a database to support studies on the natural variation and variation caused from different processing techniques in foods. The results of this project should provide for cost effective screening techniques that can be used by the food industry, FDA, other food safety agencies, and DOD to increase surveillance of the food supply for contaminants, including potential threat agents. The food industry would have "value added" incentives to apply this technology as part of their HACCP and quality assurance programs. Support for this project comes from an Army Cost-Reimbursible Research Contract to Dr. Elizabeth Calvey (Co-PI, FDA) and Dr. Bruce Jarvis (Co-PI, UM) that was initiated in August 1998.

A third year of support for Dr. Luis Rodriques-Saona was obtained. In addition, four FDA staff are involved with the development of the research - Dr. Elizabeth M. Calvey (project director), Dr. Fred S. Fry (chemometrics), Dr. Farukh M. Khambaty (microbiology) and Dr. Magdi M. Mossoba (IR spectroscopy). A fifth FDA scientist, Dr. Michael A. McLaughlin (carbohydrate chemistry) became involved to facilitate the comparison of the FT-NIR method for quantifying sugars in fruit juices with traditional HPLC methods.

(1) Methodology was developed and evaluated for the rapid detection of threat agents (castor bean meal) in foods by using Fourier-transform near-infrared (FT-NIR) spectroscopy and multivariate data analysis techniques. Measurements were made on a FT-NIR system using a diffuse reflection-integrating sphere. The manuscript for this work is currently in press. Work is continuing on refining the predictive ability of the method by incorporating water activity data.

(2) Different NIR detection devices and simple preparation methods were tested by using model solutions to determine their analytical performance. An outcome of this evaluation was the development of a simple analytical procedure for the rapid determination of individual sugars in fruit juices. In addition preliminary data has shown that sugar profiles change as a result of microbial growth. A study to determine if changes in juice sugar profiles can be used as an indirect screening method for microbial contamination will be conducted as part of subproject three below. Aqueous solutions of sugar mixtures (glucose, fructose and sucrose; 0-8% w/v) were used to develop a calibration model. Direct measurements were made by transflection using a reflectance accessory, by transmittance using a 0.5-mm cell, and by reflectance using a fiberglass paper filter. FT-NIR spectral data were transformed to the second derivative. Partial least squares regression (PLSR) was used to create calibration models that were-cross validated (leave-one-out approach). The prediction ability of the models was evaluated on fruit juices and compared with HPLC and standard enzymatic techniques. Models generated from transmittance spectra gave the best performance with standard error of prediction (SEP) $<0.10\%$

and R^2 of 99.9% that accurately and precisely predicted the sugar levels in juices. FT-NIR spectroscopy allowed for the rapid, accurate and non-destructive analysis of sugars in juices and could be applied in quality control of beverages or to monitor authenticity or contamination. This work was presented at the IFT Annual Meeting and a manuscript is in preparation.

(3) Methodology for the rapid detection of bacterial contamination in liquids was evaluated. The complex biochemical composition of bacteria yields FT-NIR vibrational transitions (overtone and combination bands) that can be used for classification and identification. Bacterial suspensions (*E. coli* HB101, *E. coli* ATCC 43888, *E. coli* 1224, *Bacillus amyloliquifaciens*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Listeria innocua*) were filtered to harvest the cells and eliminate the matrix, which has a strong NIR signal. FT-NIR measurements were done using a diffuse reflection-integrating sphere. Principal component analysis (PCA) showed tight clustering of the bacterial strains at the information-rich spectral region of 6000-4000 cm^{-1}. The method reproducibly distinguished between different *E. coli* isolates and conclusively identified the relationship between a new isolate and one of the test species. This methodology may allow for the rapid assessment of potential bacterial contamination in liquids with minimal sample preparation. The manuscript for this work has been submitted to *J. Agric. Food Chem.* Future work involves studies on detection limits, the effect of growth phases on the variability of the bacterial model, and the effect of the matrix on the bacterial identification capabilities of the model.

Publication Status:

Publications

L. E. Rodriguez-Saona, F. S. Fry, and E. M. Calvey. "Use of Fourier Transform Near-Infrared Reflectance Spectroscopy for Rapid Quantification of Castor Bean Meal in a Selection of Flour-Based Products." *J. Agric Food Chem* 2000 in press.

L. E. Rodriguez-Saona, F. M. Khambaty, F. S. Fry, and E. M. Calvey. "Rapid Detection and Identification of Bacterial Strains by Fourier Transform Near-Infrared Spectroscopy." submitted for publication *J. Agric. Food Chem.*

Presentations

L. E. Rodriguez-Saona, F. S. Fry, Farukh M. Khambaty, and E. M. Calvey. "FT-NIR Rapid Determination of Food Contaminants." EAS, 1999, Somerset NJ.

L. E. Rodriguez-Saona, F. M. Khambaty, M.M. Mossoba, F. S. Fry, and E. M. Calvey. "FT-NIR Rapid Determination of Food Integrity." USDA Biosensor Meeting, Beltsville, MD. Feb 2000.

L. E. Rodriguez-Saona, F. M. Khambaty, M.M. Mossoba, F. S. Fry, and E. M. Calvey. " Detection and Classification of Bacteria Strains by Fourier Transform Near-Infrared Spectroscopy." 2000 FDA Science Forum. Washington, DC. Feb 2000.

L. E. Rodriguez-Saona, F. S. Fry, and E. M. Calvey. "Rapid Analysis of sugars in fruit juices by FT-NIR: Comparison of Sampling Devices." IFT Annual Meeting, Dallas, TX. June 2000.

Development of Core Facilities:

The development of core facilities that will benefit FDA and University scientists and their collaborators is a cornerstone of JIFSAN's cooperative programs and objective to leverage resources. Dedication of an electron microscopy facility jointly supported by the FDA and the University of Maryland occurred during the year. The existing facility was renovated (UM-FDA joint funding) and houses four University of Maryland microscopes in addition to a new electron microscope purchased by the FDA. The facilities are staffed and used by scientists from both organizations. FDA has moved its nuclear magnetic resonance instrumentation and personnel to the NMR facility in the Chemistry building and is providing major funding to upgrade its 400 MHz instrument. Space that will house CFSAN's research mass spectrometry facility is being provided in a new addition being constructed for the Department of Chemistry and Biochemistry. The effective use of this arrangement will increase when CFSAN relocates to its new facilities in College Park in the latter half of 2001.

Risk Analysis

Risk analysis applied to food safety is the assessment, management, and communication of risks associated with our food supply. Risk analysis requires multidisciplinary input to identify, analyze, and ultimately guide the development of science-based policies. JIFSAN has identified risk analysis as a major area of focus.

JIFSAN has been charged with the responsibility of developing and operating a Risk Assessment Clearinghouse (Dr. W. Hueston, UM, Director; Dr. Wendy Fineblum, Coordinator). In 1999, the name was changed to the JIFSAN Food Safety Risk Analysis Clearinghouse to more closely align with international nomenclature in which risk analysis is the umbrella term that includes risk assessment, risk communication, and risk management. The Clearinghouse is being established to collect and disseminate available data and methodologies from government, academic, and industry sectors domestically and internationally. The Clearinghouse will provide a centralized information source for risk analysis (assessment, management, and communication) related to food safety with initial emphasis on microbial pathogens and their toxins. The Clearinghouse is guided by the Food Safety Initiative (FSI) Risk Assessment Consortium (RAC) composed of a representative from each of the government agencies responsible for ensuring the safety of the food supply.

Progress this year included:

- Creation of the Clearinghouse website at www.foodriskclearinghouse.umd.edu. Input from previous public meetings and expert workshops was used to set priorities in creating the site. Additional input is continually solicited for improvement of the Clearinghouse.

- Dr. Charlie Yoe, an economist who is active in risk analysis, joined the Clearinghouse staff as a visiting scientist in February 2000 and will remain until the end of the year. In addition to contributing to the development of the strategic plans for the Clearinghouse and JIFSAN and to content for posting on the website, he has utilized the Clearinghouse in many of the food safety risk analysis courses in which he has been involved globally.

- Dr. Wendy Fineblum gave an invited presentation on the Clearinghouse at the Toxicology Forum - Europe in Brussels, May 11.

- Food Safety Risk Analysis: Creating an Internet Community, University of Maryland at College Park, August 23 for 22 participants was organized by Dr. Wendy Fineblum and sponsored by the Clearinghouse. The meeting had two main objectives: a) to foster communication among various groups with an interest in posting food safety risk analysis resources, and b) to identify resources that are needed to create a community of food safety risk analysts and scientists on the internet, as well as the challenges that are faced in presenting these resources. Following five presentations on resources available on the internet for food safety risk analysts and one presentation on user needs, four discussion groups developed a listing of resources needed to create a viable internet community of food safety risk analysts. A summary of this meeting has been posted to the Clearinghouse website.

- The Data Evaluation Working Group, with representation from government, industry, and academia, has met since January to establish guidelines for data submitted for posting on the Clearinghouse. Instead of a formal review process, the data will have to meet minimal quality standards and be accompanied by detailed information.

- Items posted to the Clearinghouse website (http://www.foodriskclearinghouse.umd.edu) include:

    1. The Audits International/FDA Food Temperature Databases. The data were collected by over 900 specially-trained consumers. The Food Temperature Database tracks refrigerated and frozen food temperatures from the retail source during travel and to the home refrigerator or freezer. The Cooking Temperature Database demonstrates final cooking

temperatures for a range of hot foods prepared in the home. These important data have never before been available publicly.

2. Data needs page where those planning a risk assessment can request data from the Clearinghouse's global audience

3. On-line user needs survey to get feedback from users so that their needs can be better addressed

4. Risk Management - Economic Factors, Decision Criteria: a comprehensive list of links to websites that offer relevant economic information, and a document describing general economic decision criteria that can be applied to risk analysis

5. The Risk Assessment Consortium website - hosted by the Clearinghouse. This interagency group represents 18 U.S. government agencies involved with food safety.

6. Consumer Food Safety links: several websites are listed to serve individuals who find the Clearinghouse website while seeking useful resources. Consumer information is an important component of risk communication and, thus, is relevant to the Clearinghouse.

7. Terminology: the Clearinghouse provides links to the websites of various groups that provide a range of definitions used in risk analysis.

Education and Outreach Programs

The establishment of domestic and international education and outreach programs is of vital importance to JIFSAN. These programs involve aspects of food safety, applied nutrition, animal health sciences, and risk analysis that have been identified as areas of need within the purview of JIFSAN's responsibilities. Identification of these areas is done in collaboration with the JIFSAN Advisory Council. The following efforts were initiated or continued during the reporting period.

JIFSAN Website: This comprehensive website, listing JIFSAN activities in addition to a description of its program and mission, has been updated and modified by Ms. Wendy Buckler. The address is http://www.jifsan.umd.edu. This website is linked to that of the Risk Analysis Clearinghouse.

JIFSAN at IFT: For the fourth consecutive year, JIFSAN staffed a booth at the Food Exposition at the Institute of Food Technologists' Annual Meeting. This meeting had an attendance of over 17,000. Many food scientists, nutritionists, and industry representatives had an opportunity to visit with personnel from JIFSAN and to become better acquainted with the JIFSAN programs and mission.

Two adjoining booths were staffed in cooperation with personnel from the National Center for Food Science and Technology.

JIFSAN at the National Association of State Universities and Land Grant Universities (NASULGC) Exhibition: JIFSAN staffed a booth at the NASULGC Third Annual Agricultural Research and Education Exhibition and Capitol Hill Reception in the Rayburn House Office Building on March 7, 2000. The purpose of this exhibition was to inform Congress (House and Senate members and their staffs) of the benefits of agricultural research and education and showcase some of the exciting cutting-edge science taking place in this arena in the Land-Grant System. Information featuring some of JIFSAN's programs in food safety and the Food Safety Risk Analysis Clearinghouse was featured.

International Training Program "Enhancing the Safety of Fresh Produce at the Source through Good Agricultural Practices": This five-day training/information exchange program, cosponsored by JIFSAN and the FDA, was offered in Santiago, Chile, June 5-9, 2000. This program involved approximately 50 individuals from government, industry, and academia in Chile in an information exchange concerning good agricultural practices in the safe production of fresh-cut vegetables and fruits for export to the U.S. It represented the fore-runner of two pilot programs in Trindad/Tobago and Brazil to be offered by JIFSAN and the FDA based on an international training manual being developed through a subcontract to the University of Arkansas. Evaluations by participants indicated that the training program was successful and one of the best in the last decade from their viewpoint.

A draft copy of the training manual "Improving the Quality and Safety of Fresh Fruit and Vegetables: A Training Manual for Trainers," prepared by the FAO Center for Food Quality, Safety, and Nutrition, Institute of Food Science and Engineering, University of Arkansas, was received in August. It is undergoing technical review by a panel selected by the University of Arkansas. Further evaluation will occur during the two pilot programs indicated above. Results of the review and pilot programs will be used in revising the manual before final acceptance by JIFSAN.

Conferences and workshops:

1. Fumonisins Risk Assessment Workshop, January 10-12, 2000, Inn and Conference Center, University of Maryland at College Park. The meeting was cosponsored by JIFSAN, the FDA, USDA, and the World Health Organization (WHO). Approximately 120 scientists, risk assessors, and regulators from several different nations participated in this open forum for discussion of issues related to the occurrence in corn and the toxicity of the fumonisin mycotoxins. The objectives of the workshop were to explore risk assessment methods; obtain information needed for risk assessments, including the identification of

important data gaps; and review risk management implications and options.

2. Analytical Techniques for Allergenic Residues, Inn and Conference Center, University of Maryland University College, April 27-28. Cosponsored with the Food Allergy Research and Resource Program (FARRP), University of Nebraska – Lincoln. Approximately 70 individuals participated in this conference that covered the basics of food allergy, perceptions of food-allergic consumers, how regulatory agencies are addressing the allergen issue, what methods are available now and which are in development, what applications and pitfalls of these methods have been encountered by industry to date, detection limits vs clinical thresholds, and the future of allergenic residue analysis.

3. CSL/JIFSAN Joint Symposium on Food Safety and Nutrition: Risk Assessment and Communication in Food Safety, Central Science Laboratory (CSL), Ministry of Agriculture, Fisheries, and Food (MAFF), York, UK, June 20-22. Approximately 90 individuals participated in this symposium that was organized to provide those involved in different aspects of risk analysis the opportunity to meet and discuss both chemical and microbiological risk assessments and risk communication. Topics included risk analysis and the law, with an emphasis on international aspects; the internationally increasing significance of risk assessment in human safety evaluations; the type of data used in risk assessment, including human data from dietary exposure to phthalates, ochratoxin A, caffeine, dioxins, and predictive testing for allergy in novel foods; microbiological studies of *Listeria monocytogtenes, Campylobacter jejuni,* and microbial resistance to antibiotics; and the communication of risk assessment from both regulatory and industrial viewpoints. Papers from the symposium are to be published in "Food Additives and Contaminants."

4. Valuing the Health Benefits of Food Safety, Inn and Conference Center, University of Maryland University College, September 13-15, 2000. Sponsors included JIFSAN, The Centers for Disease Control and Prevention (DHHS), Economic Research Service (USDA), Environmental Protection Agency (DHHS), Food and Drug Administration (DHHS), Food Safety and Inspection Service (USDA), NE-165 Regional Research Project, and Office of the Assistant Secretary for Planning & Evaluation (DHHS). Approximately 80 participants were involved. The conference served as a first step toward generating a consensus on the current state of knowledge and deciding on a common approach to valuing the benefits of improvements in food safety. Subjects discussed included: a) how to estimate the value of a statistical life, b) how to reconcile or compare

stated willingness-to-pay (contingent valuation), revealed willingness-to-pay (hedonic measures), and cost-of-illness estimates of the value of reducing foodborne illness, c) measuring (including comparative measures) the value of reducing bacterial, chemical and other hazards, and d) measuring the pain and suffering and indirect productivity losses associated with foodborne illness.

Seminars co-sponsored by JIFSAN:

1. Dr. Richard Daniels (Audits International), "Changing Home Safety Practices: Evolution or Revolution," cosponsored with Graduate Programs in Food Science and Nutrition, Department of Nutrition and Food Science, University of Maryland, October 14, 1999.

2. Dr. John Herrman (WHO Joint Secretary of JECFA), "Evaluations of Food Additives, Contaminants, and Veterinary Drugs by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and their Role in Codex Standards," cosponsored with CFSAN, FDA, March 30, 2000.

3. Dr. Donald Zink (Director, Food Safety, Nestle USA), "HACCP as a Tool for Pathogen Control: A Case Study," cosponsored with CFSAN, FDA, April 25, 2000.

4. Professor Laurie Hall (Herchel Smith Laboratory for Medicinal Chemistry, Cambridge University, UK), "Quantitation of Food Quality and Processing by Magnetic Resonance Imaging," cosponsored with CFSAN, FDA, May 24, 2000.

5. Dr. Gerald G. Moy (Acting Coordinator, Programme of Food Safety, Department of Protection of the Environment, WHO, Geneva, Switzerland), "Exposure Assessment of Mycotoxins – A Global Perspective," cosponsored with CFSAN, FDA, August 2, 2000.

Visiting Scientists under the auspices of JIFSAN:

1. Dr. Fritz Kaferstein (WHO retired) is spending three years (November 1998 – November 2001) as a JIFSAN Distinguished Visiting Scientist. His time is equally split between the FDA and USDA advising on issues of risk analysis (risk assessment, risk communication, and risk management).

2. Mr. Katsuichi Himata (Yamazaki Bakery, Tokyo, Japan) is spending a year as a JIFSAN visiting scientist in the laboratories of the Division of Product Manufacture and Use, FDA. In support of the industry's efforts to reduce bromate residues to less than 20 ppb in finished

products, Yamazaki Bakery has authorized Mr. Himata to work on the development of a quick test for bromate residues in baked goods. Such a technique is of mutual interest to the FDA and the baking industry. Successful accomplishment of this goal will expedite surveillance responsibilities of the FDA and will permit inexpensive analyses of finished products as part of an industry-wide GMP or HACCP program.

International Cooperation and Visitors:

1. Dr. Wesley Long (FDA JIFSAN Liaison Staff) participated as an "invited" expert in a World Health Organization (WHO) planning meeting on microbiological risk assessment in Geneva, Switzerland in October 1999

2. Dr. David Lineback met with Dr.Martine Hirsh, Managing Director of the French Food Safety Agency (created in April 1999) at an evening dinner arranged by Paul Mennecier, Deputy Counselor for Agriculture, Embassy of France. Also in attendance were Dr. Lester Crawford (Center for Food and Nutrition Policy, Georgetown University), Dr. Caroline Smith DeWaal (Center for Science in the Public Interest) and Ms. Nancy Donley (Safe Tables Our Priority). Discussions involved issues of food safety from the perspective of France and the four organizations represented at the dinner.

3. Dr. David Lineback served as chair for Session II "Risk Assessment" at the International Conference: Risk Analysis and Its Role in the European Union sponsored by the European Commission in Brussels, July 18-19.

4. Dr. David Lineback met with Dr. Bernard Chevassus-au-Louis, President, French Food Agency at the Embassy of France, July 21. Dr. Chevassus-au-Louis requested this meeting the day after the Embassy of France hosted the Europe-USA Seminar "Why a Precautionary Principle in Food Safety?" The discussion ranged from issues surrounding the precautionary principle to food safety issues faced in France and the U.S. and the two organizations involved in the meeting.

5. Dr. Samuel Page, Associate Director, JIFSAN met with Dr. Sun-Hee Park, Chief Research Scientist, Korea Food and Drug Administration, August 23. Discussions centered on bioengineered foods. Dr. Park had been assigned the task of developing a policy for foods derived from GMOs.

6. Dr. Samuel Page met with Dr. Liu Xiumei, Deputy Director, Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive

Medicine on August 24. Discussions explored mutual interests in developing collaborative research projects in the areas of mycotoxins - analytical methods, food survey data, molecular epidemiology, including nutritional factors; genetically-modified organisms - data for safety assessments, validation of methods for detection; and traditional Chinese medicines - compositional data and safety determinations of functional foods based on traditional Chinese medicines.

7. Mr. Sik-yiu Kwan, Chief Chemist, Government Laboratory, Hong Kong visited with personnel in CFSAN, CVM, and the University of Maryland, September 5-6. Subjects discussed included genetically-modified organisms (GMOs) - labeling and analysis, veterinary drug residues in foods -control and legislation in a global prospective; and other food safety issues of mutual interest.

JIFSAN Student Internship Program

The JIFSAN Student Internship program is designed to provide University of Maryland undergraduate and graduate students with an opportunity to collaborate with FDA scientists on specific projects related to the JIFSAN mission. This program was implemented as part of the agreement between the University and FDA to cooperate in educational efforts. These opportunities for students enhance their knowledge of and experience in science, particularly in a regulatory environment, and familiarize them with career opportunities in the regulatory sector of public service. These intern positions may be part-time during the semester and full-time during the summer. Students' participation in the program requires that they be entering their sophomore year and majoring in such disciplines as Biology, Microbiology, Biochemistry, Chemistry, Food Science, Entomology, and Animal Science. In addition, during the initial internship semester the student is a volunteer intern working on a specific project. Upon successful completion of the initial volunteer internship, students can be paid for further work in the project. During all phases of the internship, the students have a variety of ways to obtain academic credit for their internship experience.

Twenty-nine (29) University of Maryland students have interned in CFSAN or CVM laboratories in the last year (September 1999 – August 2000). Sixteen (16) of those students were new to the program. Students who volunteered for at least one semester were given the opportunity to apply for a paid JIFSAN student position for subsequent semesters. Twenty (20) students were paid for at least one semester of their internship at the FDA. Eleven (11) students were paid to work a minimum of thirty hours per week at CFSAN or CVM labs during the Summer 2000. The website listing internships is at http://www.life.umd/edu/jifsan/internships.htm.

Participation in the JIFSAN Internship Program continues to grow. The UM Office of Science Outreach and Special Programs has worked to increase student, faculty, and staff awareness of the Program through its literature, seminar series, and the annual Internship Day which it sponsors. The JIFSAN Program has been well represented at the Internship

Day by Ms. Wendy Buckler, the JIFSAN Program Specialist at the FDA. One of the strongest features of the Program is the continuing collaborative spirit among the participants.

JIFSAN Student Interns: Fall 1999 - Summer 2000

Rahim Curtis (Cell Molecular Biology and Genetics major, College of Life Sciences), is working with Dr Keith Lampel on the development of a universal extraction of foods for a Polymerase Chain Reaction (PCR) based assay for the rapid identification of food-borne pathogens. Rahim will use several representative food groups to develop washing and/or extraction methods that will efficiently isolate DNA templates from contaminating microbial pathogens and then adapt these protocols to identify isolated organisms in the washes or extracts. Started 1/99. *Paid Summer '99, Volunteer Fall '99.

Lale Evens (Microbiology major, College of Life Sciences) has worked with Pat Rogers on developing a method to determine products of decomposition in crabmeat by FID Gas Chromatography and a comparison of food-borne and clinical isolates of *Listeria monocytogenes*. Started Summer '99. Paid Fall '99

Cassandra Grenade (Cell Molecular Biology and Genetics major, College of Life Sciences) is working with Dr. Maryann Principato on a project to characterize the immunologic response of T lymphocyte populations in the mammalian gastro-intestinal tract. Started 6/2/98. *Paid Fall '98, Spring '99 non-FSI, Summer '99, Fall '99, Spring '00 and Volunteer Summer '00.

Tabitha Harmon (Biology major, College of Life Sciences) worked with Dr. Jeffrey Yourick to study the percutaneous absorption and metabolism of the cosmetic ingredients, methyleugenol and diethanolamine. Start Summer '00.

Joshua Hayes (Graduate Student in Microbiology, College of Life Sciences) works with Dr. David Wagner on his project, " Surveillance of Poultry and Other Stock for Carriage of Multi-resistant Enterococcus". Started 4/99. CVM Volunteer Fall '99, Spring '00 and Summer '00

Laudan Izadi (Biology major, College of Life Sciences), is working with Dr Marianne Milliotis on identification and characterization of virulence determinants *for Vibrio Parahaemolyticus*. The student will perform mutagenesis of a hemolysin-negative toxin producing strain *of v. parahaemolyticus* by conjugation of the vibrio with a transposon-containing *E.coli* strain. Started 1/99. *Paid Summer '99, Summer '00

Alex Karamian (Microbiology major, College of Life Sciences) is working with Dr. Alan Olsen on a project to evaluate the significance of house flies, as an emerging vector of *Salmonella.* He will manage cultures of *Salmonella* , assist in

inoculation of house flies and evaluate final culture plates to enumerate *Salmonella* transfer by flies. Started 6/99. Volunteer Fall '99.

Kyu Won Kim (Cell Molecular Biology and Genetics major, College of Life Sciences) worked with Dr. Ken Ku on his analysis of carbohydrates in foods and research on conjugated linoleic acid. Started WT-Spring '00. Paid Summer '00

Saeeda Latham (Biochemistry major, College of Life Sciences) worked with Dr. Maryann Principato on her project "Immune Response in the Gastrointestinal tract: Staphylococcal enterotoxin". Started Spring '00. Paid Summer '00.

Esther Lazar (Dietetics major, College of Agriculture and Natural Resources) works with Dr. Ken Ku studying issues related to Dr. Ku's project, "Dietary Fiber in Foods". She assists in routine techniques such as analysis of various food fiber types and is helping with the development of new methods for separation of specific fiber components. In the summer of 2000 she began working with Dr. Nega Beru on the development of a regulatory egg-traceback database. Started 6/99. Paid Fall '99, Spring '00, Summer '00.

John Lee (Microbiology major, College of Life Sciences) is working with Dr. Keith Lampel on the "Development of a Universal Extraction Method of Foods for a Polymerase Chain Reaction (PCR)-based Assay for the Rapid Identification of Food-borne Pathogens". Methods will be tested by spiking representative food samples with known pathogens currently associated with food-borne illness. Started 1/99. *Paid Summer '99. Volunteer Fall '99. Paid Spring '00, Summer, '00.

Ann McCarthy (Biology major, College of Life Sciences) is working with Dr. Richard Whiting, Ph.D. on modeling the thermal inactivation of *Listeria moncytogenes*. Started 1/99. *Paid Summer '99, Fall '99, Spring '00, Summer '00.

George Mehen (Cell Molecular Biology and Genetics major, College of Life Sciences) worked with Dr. Ben Tall on his project "Signal Transduction Mechanism Involved in the Adherence and Invasion of Vibrio vulnificus. Started Spring '00. Paid Summer '00.

Jennifer O'Driscoll (Non-degree seeking, College of Letters and Sciences) worked with Dr. Bader Shaikh on the establishment of withdrawal time for furosemide, a diuretic drug used in dairy cows. Started 1/99. Volunteer Spring '99 and Summer '99, Fall '99.

Phare Okelo (Graduate Student in Biological Resources Engineering, Colleges of Life Sciences and Engineering) worked with Dr. David Wagner on his project, "Surveillance of Poultry and Other Stock for Carriage of Multi-resistant

Enterococcus". Started 4/99. Volunteer Spring '99. Paid Fall '99, Spring '00, Summer '00. CVM

Roger Plaut (Microbiology major, College of Life Sciences) worked with Dr. Keith Lampel on a project to improve detection of enteric pathogens by PCR. Started Summer '00

Kristen Pulio (Biochemistry major, College of Life Sciences), is working with Dr. Kim Moorehouse on food irradiation safety issues. The student will use super critical fluid extraction or accelerated solvent extractor for the isolation of radiolytically generated products formed during the irradiation of the lipid present in foods, and to utilize these radiolytic products as markers for irradiation treatment of food. The radiolytic products will be monitored and identified using gas chromatography with either flame ionization detection or mass spectroscopic detection. Started 4/99. *Paid Summer '99, Fall '99. FSI.

Shahikala Ratnayake (Biochemistry and Microbiology major, College of Life Sciences) is working with Dr. Robert Hall on the purification and characterization of a novel cytotoxin of *Vibrio cholerae.* The student will develop skill and confidence in microbiological experimentation through safe handling of micro-organisms. These skills will contribute to an ongoing project to purify a virulence determinant from *Vibrio cholerae* . Started 2/12/99. *Paid Summer '99, Fall '99.

Monica Ruiz (Microbiology major, College of Life Sciences) is working with Dr. Maryann Principato studying the "Immunologic Effects of *Staphylococcal enterotoxins"*. Started Summer '00.

Jilla Shahnematollahi (Biochemistry and Neruophysiology major, College of Life Sciences) is working with Dr. Ian DeVeau on the predictability of the transfer of drugs and metabolites from plasma into milk of humans and animals. She will assist in extractions and analysis of plasma and milk samples. She will also help develop and validate analytical methods for the determination of enrofloxacin and fentanyl. Started 6/99. Paid Fall '99. Contract Spring '00 and Summer '00.

Alexander Shangraw (Microbiology major, College of Life Sciences) is working with Dr. Ben Tall on emerging pathogens in seafood. The student will be involved in many aspects of the project which involves purification of fibrillae, production of monoclonal antibodies and invasion assays. Started 1/20/99. *Paid Summer '99, Fall '99 and Spring '00.

Bhavana Sharma (Physiology and Neurobiology, College of Life Sciences) works with Dr. Dan Levy investigating the properties of *E. coli* and *Salmonella* strains and comparing them with the mutator phenotype. She is learning and using techniques of microbiology and molecular biology to characterize the way these bacterial strains acquire resistance to antibiotics used in agriculture and

medicine. Her work centers on the response of mutator strains to clinical assays for antibiotic resistance, the genes responsible for anomalous responses to the field assays, and other investigations of the properties of these antibiotic resistant bacterial strains. Started 6/99. FSI graduate stipend for this work. Started 6/25/97. *Paid as undergraduate Summer and Fall 98, paid as graduate student Spring '99 and Summer '99, Risk Assessment. Paid Fall ’99 and Summer ’00.

Coryse St. Hillaire (Biochemistry major, College of Life Sciences) worked in the laboratory of Dr. Geraldine Mitchell on her ongoing project: Analytical and Biological Evaluation of Selected Food Products. Started Fall ’99. Paid Spring ’00 and Summer ’00.

Nolawi Taddesse (Microbiology major, College of Life Sciences) worked with Dr. Robert Hall on the continuing work in his laboratory regarding the detection of pathogens. Start Summer ’00.

Phoebe Thangawng (Microbiology major, College of Life Sciences) worked with Dr. Maryann Principato to study the “Immunologic Effects of Ingested Staphylococcal Enterotoxin B”. Started Summer ’00.

Michael Tims (Graduate Student in Plant Biology, College of Life Sciences), worked with Dr. Joseph Betz on the study of mistletoe lectins in the initiation of pathogenesis. He received a summer and Fall stipend for isolating and characterizing the biologically active compounds from American mistletoe using extraction, bioassay and analytical and semi-preparative High Pressure Liquid Chromatography of plant material. In Spring '99 he moved on to work with Dr. Mark Walderhaug on the Bad Bug Book. Started Summer ’97. Paid graduate student stipend Fall ’98, Spring ’99, (Risk Asses. Sup.) Fall ’99, and Spring ‘00. Contract Summer ’00.

Sabena Uddowla (Microbiology major, College of Life Sciences) worked with Dr. Robert Merker on his studies of “Acid Tolerance in Fish-borne Pathogens” and his investigation of the use of surrogate, non-pathogenic, bacteria in the testing of juice-processing plant procedures. Started Spring “00. Paid Summer “00.

Patrick Vorhees (Biology major, College of Life Sciences) is working with Dr. Robert Hall on the development of a detection system for *Escherichia coli* 0157:H7 and other enterohemorrhagic serotypes. He will learn safe handling of micro-organisms, design of experiments and genetic amplification methods. Started 1/99. Volunteer Summer '99. Fall ‘99.

Aisha Yansaneh (Microbiology major, College of Life Sciences) worked with Dr. Anthony Hitchins to continue studying the application of a newly developed method for enumerating low levels of food-borne *Listeria*. Started Fall ’98. Paid Spring ’99, Summer ’99 and Fall ’99

Future Plans (2000-2001):

Continue development and increase use of the JIFSAN Advisory Council

Continue development of educational materials and training programs for enhancing the safety of fresh produce at the source of production in countries exporting to the U.S. Training programs are scheduled for Trinidad/Tobago, December 2000, and Brazil, June 2001.

Continue internal grants program. Fund three to five new project proposals effective July 1, 2001.

Submit at least one collaborative research proposal for competitive funding.

Award a subcontract to the National Food Processors Association (NFPA) to collect data on the occurrence of *Listeria monocytogenes* in five selected foods. This is part of a larger study sponsored by USDA to obtain data to be used in determining exposure estimates for the U.S. population to *Listeria monocytogenes* in selected foods. The data from this subcontract and from the larger study will be deposited in the Food Safety Risk Analysis Clearinghouse.

Develop a cooperative education/outreach effort with the food service industry utilizing a consultant with experience in the area to help design and develop the program.

Hire a Conference and Communications Coordinator. As part of the responsibilities of this position, initiate development and distribution of a periodic newsletter.

Conduct a limited number of symposia and workshops on topics of importance to the food safety, applied nutrition, and animal health communities including:

> Giving Simple Sugars a Complex, jointly with the Center for Food and Nutrition Policy, Georgetown University, scheduled for March 15-16, 2001 at Georgetown University.
>
> Second Annual CSL/JIFSAN Joint Symposium in Food Safety and Nutrition: Current Issues in Food Biotechnology, scheduled for July 11-13, 2001, Inn and Conference Center, University of Maryland University College.
>
> Two other conferences/workshops in biotechnology are in initial stages of planning and development.

Expand operation of the Risk Analysis Clearinghouse with emphasis on the inclusion of additional risk assessment and data.

Continue development and initial offering of short courses in Plant Biotechnology and Introduction to Risk Analysis, piloting these two offerings with an FDA audience, and an internet-based course in Introductory Biotechnology.

Complete development and initiate strategic plan for JIFSAN

Continue development of international contacts and collaborative programs with an emphasis upon development of active cooperative programs with the CSL, MAFF, UK; the Department of Natural Resources and Environment, State of Victoria, Australia; the European Commission, Brussels; and the Joint Research Center (EU) at Ispra, Italy.