

DAIRY PIPELINE

From genes to cheese flavor—gene sequencing helps to fill in the blanks

It was only one year ago that Francis Collins, head of the Human Genome Project, and J. Craig Venter, head of Celera Genomics, announced that they had completed the first survey of the human genome. Since then we've learned that humans have far fewer genes than most people expected, these genes are very similar in all of us humans, and now we can locate a gene linked to a trait even though we may have no clue how that gene influences the trait. Although sequencing the human genome has gotten the most publicity, the genome story is much bigger than the human chapter.

Genomic research, or the science based on chromosomes and the genes they contain, has fostered a revolution in biology. For example, many scientists believe the practice of medicine will change as diagnosis and treatment becomes tailored to the individual patient. Agriculture will change (see sidebar on page 3) and genomics is going to influence the dairy industry, too.

Sequencing the entire string of human DNA was finished ten years earlier than projected, partly because new techniques were developed as the project progressed. However, increased collaboration among scientists is another factor that has helped to foster the genomic revolution. A current research project, funded by both Dairy Management, Inc. and Chr. Hansen, Inc., is a good illustration of both collaboration and application of genomic techniques in the dairy sphere. Jeffery Broadbent, associate professor at Utah State University, and Jim Steele, professor at the University of Wisconsin-Madison are working with the Genome Center of

Wisconsin to determine the sequence of the *Lactobacillus helveticus* CNRZ32 genome. They expect the map of *Lb. helveticus* CNRZ32 to be approximately 2.4 million base pairs (Mbp), and from this map they expect to assemble a database of CNRZ32 genes that influence the flavor of cheese.

Clues about chemistry

Decades of cheese flavor research have supplied clues about the chemistry of cheese, and without this background the link between gene and cheese flavor would be much more difficult to make. *Lb. helveticus* CNRZ32 is one of a variety of lactobacilli used by the dairy industry to intensify and accelerate cheese flavor. We know the operatives behind this influence—proteins, amino acids and lipids. We know some of the operation, or metabolism, too. Proteins, like casein, are broken up by enzymes—supplied by milk, chymosin, and bacteria. These pieces of protein are now configured as peptides and amino acids that influence both desired cheeses flavor and bitter off flavors. Of course, this simplifies the process. It gets complex quickly when you look closer at casein and the chemistry of the enzymes, or proteinases, that break it apart to produce peptides and amino acids. For several years Steele and his research group have been looking closely at *Lb. helveticus* CNRZ32, identifying several enzymes that have essential roles in the pathway that produces cheese flavor compounds. Sequencing *Lb. helveticus* CNRZ32 will fill in the blanks, locating genes for the pathways we already know and, most likely, uncovering genes for enzymes that haven't been identified yet.

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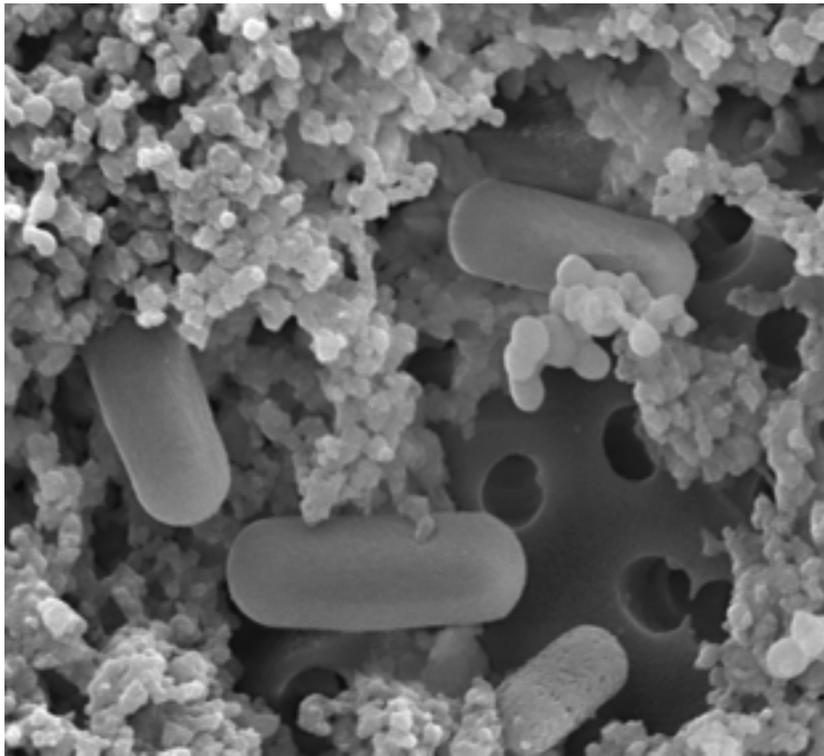
Jeff Broadbent explains that following the trail from enzyme to gene in the past was slow and tedious. "Fishing out a gene of interest can be laborious. Even more frustrating is when we know the gene is there, we know it is active, but we can't find it."

Jeff Broadbent explains that following the trail from enzyme to gene in the past was slow and tedious. "Fishing out a gene of interest can be laborious. Even more frustrating is when we know the gene is there, we know it is active, but we can't find it." Now, new tools and techniques allow him to organize and identify the sequence of genes with a laptop computer.

From gene to cheese

So how will sequencing the CNRZ32 gene lead us to information about cheese flavor? When scientists track the relationship between genotype and phenotype, or the genetic code and the physical trait the code produces, they rely heavily on past research, particularly model organisms that have powerful sets of data behind them. For example, in the plant world *Arabidopsis thaliana*, a member of the mustard family, has been the poster child for plants and the focus of botanists all over the world as they worked to sequence and decode its genes. *Haemophilus influenzae* is the star in the world of bacteria.

In 1995 it was the first independent organism sequenced, and this effort opened the door to identifying genes and the beginning of understanding how they work. Now, the Wisconsin Genome Center has already sequenced *Escherichia coli*, strains 0157:H7 and K12 and is working on a dozen other sequencing projects. Because of the earlier genetics work with bacteria, including *H. influenzae*, scientists already know the function of many identified bacterial genes. Thus, by comparing homologous regions (those areas with the same genes arranged in the same order), in newly sequenced organisms they have a road map to follow. They already know where to look for the parts of the gene they are interested in. When Broadbent and Steele put together the map of CNRZ32, they can find these similar regions and follow the map to match them up with the metabolic pathways they want to investigate. For example, they will know where to find the



An electron microscopy photo of *Lb. helveticus* CNRZ32, courtesy of Bill McManus and Utah State University.

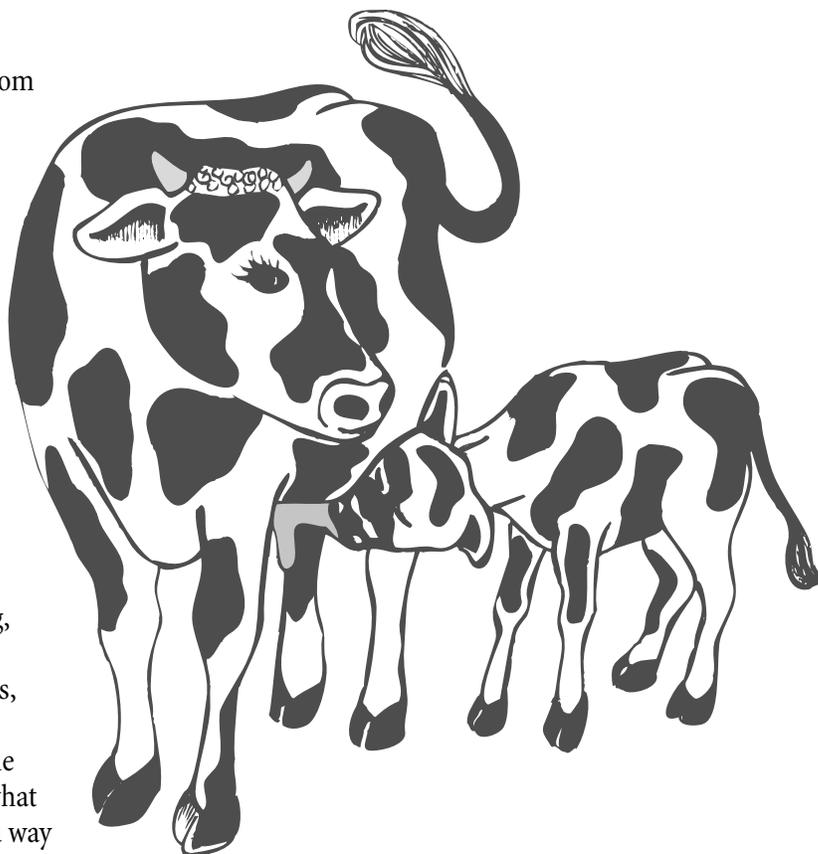
genes that code for the enzymes involved in the proteolytic breakdown of casein, and then they can design experiments that will show how the pathway contributes to cheese flavor. In the future, they will be able to compare genes in CNRZ32 to other species of *Lactobacillus*, and perhaps correlate gene action with metabolic pathway and then particular flavor notes.

Broadbent notes that genomics has had, and will continue to have a powerful influence on the way research is done. These days, "It just has to be part of the way we do science." ☺

Genomics and Agriculture

Although common in the United States, genetically modified foods have been rejected by many people in Europe and Japan. The controversy surrounding modified foods continues, influencing international trade, straining foreign relations and costing farmers thousands of dollars. Gene research offers an alternative by providing scientists with knowledge about genes that they can use to speed up and enhance conventional breeding of crops. This enhanced breeding approach allows scientists to select the genes they are interested in by using genetic markers as a guide, which can save lots of time. Although marker assisted selection has been around for at least a decade, it hasn't really lived up to expectations. However, new techniques adapted from the DNA testing methods developed for medical diagnosis will likely speed up the process of linking traits to markers allowing agricultural researchers to tap into the breeding of plants powered by genomics.

Wisconsin's dairy producers will benefit from the power of genomics, too. George Shook, University of Wisconsin dairy cattle geneticist is leading a team of researchers looking for the genetic basis of both resistance and susceptibility to Johne's disease. Johne's is a costly disease, affecting between 20 and 40 percent of US dairy herds. Johne's is also known as paratuberculosis, from *Mycobacterium paratuberculosis*, the bacterium that causes this slow-developing intestinal disease that has no treatment. Along with identifying genes that confer resistance to Johne's, genomics may point the way to more effective testing, treatment and prevention. Dr. Elizabeth Manning, one of the investigating scientists, suggests that these powerful research techniques can help us learn about both the host and the organism. "When we know what is going on then maybe we can figure out a way to interfere," she explains. That method could include developing vaccines.



Meanwhile, another team of researchers has identified the first genetic abnormality associated with a susceptibility to Crohn's disease, the human chronic intestinal disease that resembles Johne's in cows. In humans, genetic susceptibility to Crohn's disease may arise from an altered immune response to bacterial components. Essentially, the immune system in someone

genetically susceptible to Crohn's doesn't recognize the invading bacteria and thus doesn't launch an all out response. Is that bacterium the same one that causes Johne's disease in cows? We really don't know, but genomics offers new tools to help us find out. 

Interested in making Dutch cheeses?



Holland’s long history of cheese production may have begun as early as two centuries B.C.; in Friesland pots and vessels suggest cheesemaking started that early. We know that Dutch bargemen paid their tolls in cheese as they traveled down the Rhine as early as 1100 A.D.

Today, Holland remains one of the world’s largest exporters of cheese. Although most cheeses including Gouda and Massdammer, are mass produced, the Dutch still regard cheesemaking as an art form. For this reason Dutch cheeses are known around the world for their workmanship and quality.

CDR will present an artisan cheese workshop

CDR’s next artisan workshop will focus on the techniques and production methods of Dutch cheeses. After one day of lectures, attendees will gain cheesemaking experience from experts from Holland.

One of our guest cheesemakers, E. J. Osterloo, has been an instructor with the Agrarische Hogeschool Friesland – Van Hall Instituut, for over twenty years. His current book “Kaas” is used as an instructional textbook at the school. We will include an English Language version of this book with the instructional materials. Fons Michealson, our second guest cheesemaker, is a cheese making instructor at the AOC Friesland School in Leeuwarden in Holland. He is also the chairman of the European Alliance of Teachers in dairy technology.

Workshop Schedule

Day 1, Lecture Topics

A brief overview of the Dutch Dairy Industry

Starter Manufacture

Ripening techniques

Quality control

Cheese yield

Cheese manufacturing procedures

Packaging

Cheese defects

Salt brines

Mechanization

Pressing techniques

Day 2

Hands on cheese manufacture

Waxing and plasticcoating of cheese

Wrap-up discussion

For more information

This artisan workshop takes place September 25 and 26, 2001 at the University of Wisconsin—Madison. For more information about the program, call Jim Path at (608) 265-2253. To register, contact CALS Outreach Services at (608) 263-1672.

Skimming the Shelf—



What's New in Print?

The Small Dairy Resource Book

Information sources for farmstead producers and processors
by Vicki Dunaway

Vicki Dunaway's 56 page booklet is a comprehensive bibliography of resources for the dairy industry. It is geared to the small, alternative producers and processors but anyone interested in dairy products will find something here. Dunaway reviews books about cheese, ice cream, safety, animals, and feeds and grazing. Her appendix lists suppliers, consultants, courses and useful websites.

When I found Dunaway's review of *The Complete Dairy Foods Cookbook: How to Make Everything from Cheese to Custard in Your Own Kitchen* I knew she had done a thorough job. Edited by E. Annie Proulx, the Pulitzer Prize winning author of the *Shipping News*, this book was published in 1982 and is now out of print. This is still my favorite book about cheese and worth tracking down. Dunaway notes that it "is the most well-researched book of its kind that I've come across." It is still available through used book dealers, and the Internet is an ideal way to locate it. (Dunaway suggests www.bookfinder.com) Expect to pay up to \$25 for a used copy, unless you get lucky like I did when I found it at a garage sale in Madison for less than a dollar.

Dunaway's resource book was supported by the Southern Region Sustainable Agriculture Research and Education program (SARE) and can be ordered by phone, (802) 656-0484, or retrieved via the internet at www.sare.org/san/htdocs/pubs/

Cheese Moisture: Its Variations and Measurement

Special Guest Editor Section
Journal of AOAC International
March/April 2001 Vol. 84, No. 2

This is actually a group of six articles, edited by Bob Bradley, covering analytical techniques for measuring cheese moisture. Paul Kinstedt, David Barbano, David McKenna, and Doug Emmons are among the group of authors that address analytical variations by method, cheese variety and instrumentation. Contact the AOAC International to get a copy:

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Fax: 303-924-7089

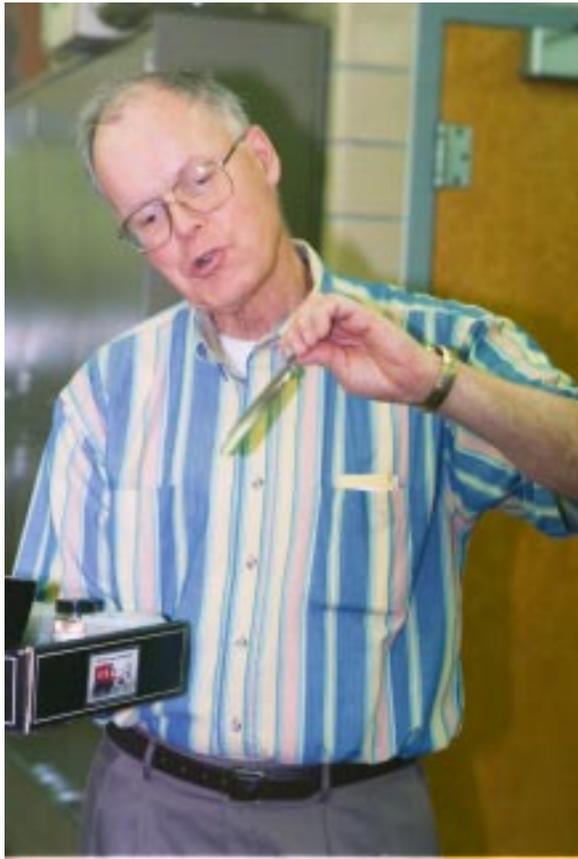
News from CDR

Professor Bradley heads out to pasture

Thirty seven years ago Robert L. Bradley, Jr. joined the Department of Food Science at the University of Wisconsin. I can't list everything he accomplished during his career at the University (it would take up the entire page), but I can tell you that he was busy. Dr. Bradley is well known for his research; he published over 100 papers and book chapters on topics ranging from cleaning and sanitation to improved methods for analyzing dairy products. His extension role has been a big part of his career, too. Many people in the dairy industry met Dr Bradley through the workshops and short courses he developed to teach them about milk pasteurization and ice cream making. Dr. Bradley's legendary role of teacher has influenced food science students for decades, in class and as the coach of the Dairy Products Judging Team. We all know he'll probably take on the role of emeritus professor rather slowly, spending less and less time at Babcock Hall. Then it will be Spring and the trout will be rising and the morels will come up. And he'll be out of here.

CDR staff in training

Carol Chen's efforts to learn more about the machinability of cheese have snared a large share of the CDR staff. We are currently training to be reproducible, repeatable and reliable instruments who can judge the texture of cheese. Carol plans to use her group of highly trained "instruments" as she investigates the characteristics of cheese and how they correlate with shredding, slicing and grinding properties during food processing.



Dr. Bradley scrutinizing his Sensory Standards for Aging Judges, a kit presented to him by Bill Wendorff at a recent cheese grading workshop. Bradley can use his kit (which packs a wallop, according to Wendorff) to refresh his taste buds on bitter flavors, horsey flavors, the essence of lipolized butter oil, and catty flavors.



Babcock Dairy Store sports a new look

You'll enjoy the same tasty ice cream that you've learned to crave at the Babcock Dairy Store, but the surroundings have changed. Reopened just this week after months of work, the store sports a cheery color scheme, a new seating area, and even a hallway to slow those cold winds blowing off Lake Mendota.

John and Donna Hansen are the benefactors behind the remodeling effort. John is a UW-Madison alumnus who grew up on a dairy farm and worked in a cheese plant before successfully launching Kwik Trip Inc. in 1965.

The Babcock Dairy Store is celebrating a week long Grand Opening, beginning with a reception on July 31, 2001. Stop in and check out the new store, a superb setting that amply displays the best ice cream in the world and Walt Brandli's fine cheeses.

Reverberations from the Food Allergen Partnership

“Equipment cleaning is a crucial control point.”

Preventing cross contamination

- ◆ Schedule production with allergen control in mind
- ◆ Be careful with common utensils
- ◆ Employee awareness and training are important
- ◆ Develop an allergy prevention plan that identifies sources of contamination and method of control

Although food allergies certainly aren't a recent phenomenon, they have certainly been a popular topic in the news lately. The Food Allergen Partnership in January 2001 drew attention to this issue when it released results of finished product surveillance samples. The project was a collaborative effort of the US Food and Drug Administration (FDA), the Minnesota Department of Agriculture and the Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP). Allergen-related recalls have increased in recent years and the Project attempted to address the increase by examining industry handling of food allergens. Although almost any food can cause an allergy, the Project focused on peanut and egg allergies, two of the eight foods that cause ninety percent of food allergies. (The rest of the lineup includes milk and milk products, wheat, tree nuts, soy, fish and shellfish.)

Routine regulatory inspections were conducted in Minnesota and Wisconsin at 13 ice cream plants, 54 bakery establishments and 18 candy manufacturers. During the process, inspectors compared raw ingredients in the formulations to the finished product labels. They found raw ingredients that were omitted from the label in twenty five percent of the plants they inspected.

In addition, ten percent of the samples they analyzed were positive for egg, and twenty-five percent of the 73 samples were positive for peanut residue.

The Project report notes that, “Food allergens may become part of a food through unintended routes.” Equipment cleaning is a crucial control point, contamination commonly occurs when clean up and sanitation are ineffective. Rework was a problem, too. Thirty seven percent of the firms used rework and of these, 40% of the MN firms and 55% of WI firms had product that was positive for undeclared allergies.

Preventing cross contamination can be relatively simple. For example, report authors suggest that manufacturers schedule production with allergen control in mind. Be careful when common utensils are used in production of allergen and non-allergen products. Some manufacturers use separate, or dedicated equipment to prevent cross contamination.

The authors of the Food Allergen Partnership report suggest that industry awareness in essential and employee awareness and training are equally important. They recommend an allergy prevention plan to identify sources of contamination and methods to control them.

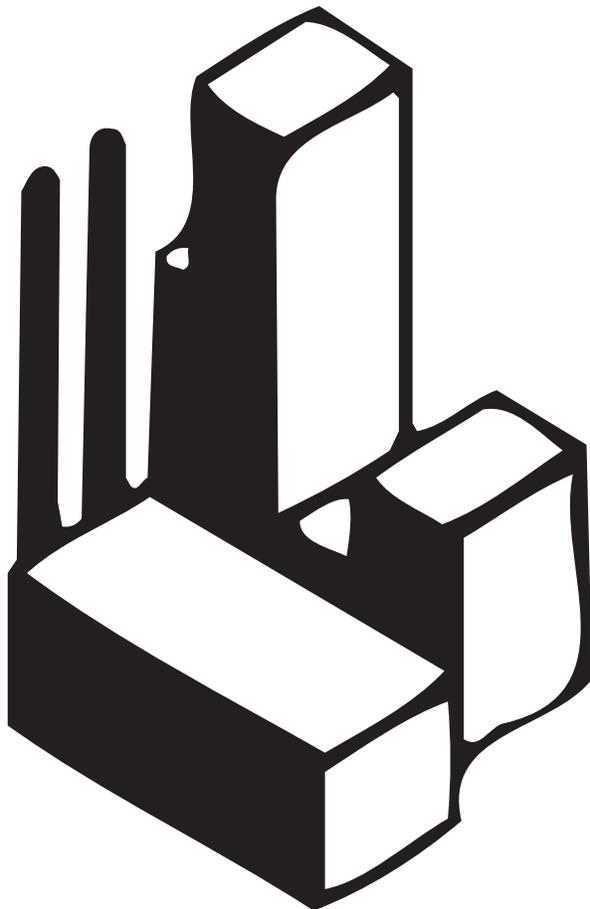


Resources

Food Allergen Partnership, report
www.cfsan.fda.gov/~dms/alrgpart.html

For labeling guidelines:
National Food Processors Association
www.nfpa-food.org
www.nfpa-food.org/science/FoodAllergenLabelingGuidelines

U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
www.cfsan.fda.gov/~dms/wh-alrgypart.html



Approximately seven million Americans have food allergies and the FDA takes its role of controlling food allergens seriously. They recently announced plans to inspect thousands of food processing plants for undeclared allergens. The food industry takes this problem seriously, too. They have worked up voluntary labeling standards that would disclose sources of flavorings that could cause allergy problems. The National Food Processors Association (NFPA) coordinates a group of food trade associations and other interested organizations, the Food Allergy Issues Alliance, that focus on allergy related issues. Along with labeling standards, NFPA is currently developing a Manual for Food Allergen Management to help the food industry adopt a Code of Practice for managing food allergens. 

An announcement from the International Food Technologists (IFT) meeting...

The US Food and Drug Administration (FDA) recently announced that it plans to follow the Midwest project with national testing. After training inspectors, the FDA plans to inspect as many as 6,000 food processing plants in an effort to prevent accidental contamination of food.

Curd Clinic

Curd Clinic doctor for this issue is Robert Bush, M.D., University of Wisconsin-Madison

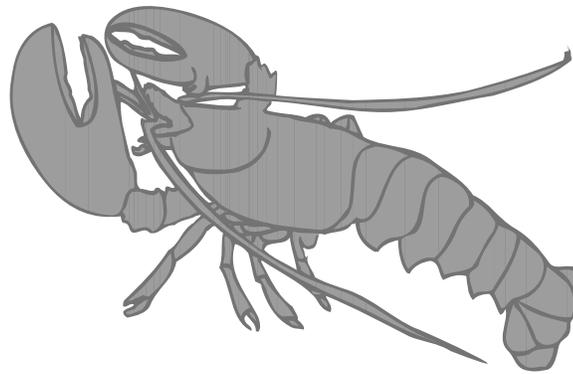
Q. We used to do a continuous run in our cold pack cheese operation, producing Swiss Almond, sharp Cheddar, and Jalapeno all in one day. Now, we have been told that we have to clean after every run because of food allergens. Could a tiny piece of almond in the sharp Cheddar really cause that much of a problem?

A. The answer to this question is yes, yes, yes! Although food allergies only affect a very small portion of the U.S. population the effect can be serious. The exact number of suspected individuals is not known, but estimates place the total prevalence at about 1-2% of the population. Food allergies are more common in children than adults and prevalence rates between 3-6% have been estimated. The most common problems seen in children are reactions to milk and eggs which may be outgrown as the child ages, often around the age of puberty. A study of hospital admissions to 73 Colorado emergency departments estimated that there are approximately 950 severe reactions to food annually in the United States. The number of fatalities as a result of food allergic reactions is not entirely known although a national registry for food allergic related deaths reports 32 fatalities between 1994 and 1999. This probably underestimates the exact numbers.

The fatalities typically affect younger individuals, from age 2 to 33 years. Peanuts accounted for 63% of the fatalities and tree nuts accounted for 10%. Two other deaths occurred from milk and fish.

Foods that cause allergies

What are some of the common foods that cause food allergies? In the U.S. population, peanuts are the most common food that causes allergic reactions across all age groups. In children, as mentioned above, milk and egg are common causes of allergies. Soybean protein may also be a problem and in older children peanuts become a significant problem. In adults, tree nuts, peanuts, fish, and Crustacea such as shrimp and lobster are common causes of severe allergic reactions.



However, over 200 different foods have been implicated as the cause of food allergic reactions.

What causes food allergy? Essentially, we all have the ability to become allergic to foods, however, only a few people actually develop sensitivities. Often, people who develop food allergies have other allergies, such as hay fever, or other allergic symptoms like asthma and eczema. These allergies do have a tendency to run in families, suggesting that there is a genetic tendency to develop allergies. Food allergies are the result of an immune reaction to specific proteins in foods. Antibodies of a particular type, called IgE, are directed against these proteins. The antibodies circulate in the bloodstream and attach to cells in the body called mast cells—located in the respiratory system, the gastrointestinal tract, and the skin. Mast cells contain histamine and other chemicals that are released when the person is exposed to the food that contains the protein that causes the allergy.

Typical symptoms from a food allergic reaction include generalized itching, the development of hives, swelling in the face, lips and throat, difficulty breathing, nausea, vomiting, and occasionally diarrhea. Victims can develop severe asthma attacks. Sometimes they develop a shock reaction, which can be fatal if untreated.

Diagnosis

How are food allergies diagnosed? Often people learn of their allergies from experience and simply avoid problem foods. Physicians can detect whether a person is allergic by performing certain tests, such as a skin test, to identify the specific food allergy. In some instances blood tests can be done, such as a radioallergosorbent (RAST) or an enzyme-linked immunoassay. These tests detect the presence of the IgE antibodies to the specific food.

How much of the food has to be eaten before reaction occurs? The exact quantity that will produce an allergic reaction in an individual is quite variable. However, studies with peanuts indicate that people who are highly allergic to peanuts will notice

objective symptoms—such as a feeling of itching or uncomfortable feeling— after very small amounts of peanut. For example, as little as 5 milligrams of peanut protein may produce reactions such as hives or swelling, diarrhea or vomiting. Thus, in the example cited at the beginning of this article, the presence of a small amount of an almond in cheese could prove very dangerous to highly allergic individuals.

Treatment

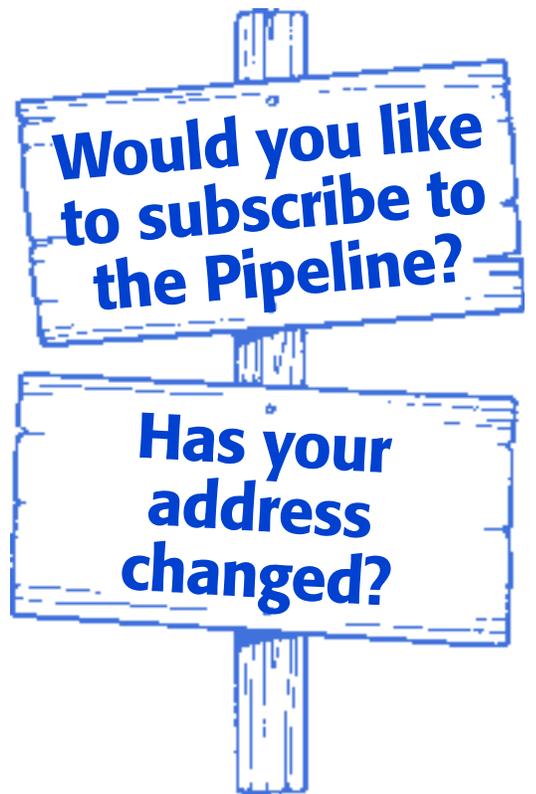
What is the treatment of food allergy? First and foremost the treatment of food allergy is actually prevention by avoiding the problem food. However, individuals with food allergies are at risk for unexpected exposures when they consume products that are not labeled as containing the food allergen. Food allergic consumers rely on the manufacturers to provide appropriate product labeling. Recent studies on food products, many of which are manufactured in Wisconsin and the upper Midwest, indicated that inadvertent contamination by food allergens was common. Therefore, it is incumbent on the manufacturer to minimize risk to food allergic individuals by proper cleaning and minimizing any risk of contamination of their product. If the product does indeed contain potential allergens, label them accordingly. New regulations state that common names should be used to avoid confusion, such as using terms like milk products in place of casein because most people do not understand that casein is a milk protein. Even when people allergic to milk realize that casein is a common milk protein, their friends and relatives may not make the connection and mistakenly reassure them about the safety of prepared food.

In conclusion, food allergies, although not common, can be lethal. Minute quantities of the food contaminating products can be a source of hazard for the food allergic individual. Consumers rely heavily upon the quality controls and safety procedures of the food processors to ensure their health, and their lives. ☺

Calendar, continued from page 12

Nov. 1-3 Great Lakes Dairy Sheep Symposium. Eau Claire, WI.
Program Coordinator: David Thomas (608) 263-4306.

Nov. 7-8 Wisconsin Cheese Grading Short Course. Madison, WI.
Call Bill Wendorff at (608) 263-2015.



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You can also find the Dairy Pipeline on our website: www.cdr.wisc.edu

Calendar

Aug. 2-5 American Cheese Society Annual Meeting. Louisville, KY. For info, call (262) 728-4458.

Aug. 21-23 Milk Pasteurization and Process Control School. Madison, WI. Call Bob Bradley at (608) 263-2007 for information, or the CALS Conference Office (608) 263-1672 to register.

Sept. 5 Managing Dairy Food Safety Workshop, Madison, WI Program coordinator: Marianne Smukowski, (608) 265-6346.

Sept. 25-26 Dutch Cheese Artisan Course, Madison, WI. Program Coordinator: Jim Path, (608) 262-2253.

Sept. 25-26 Dairy, Food and Environmental Health Symposium. cosponsored by Wisconsin Association of Milk and Food Sanitarians, WI Association of Dairy Plant Field Reps, and WI Environmental Health Assn., Wisconsin Dells, WI. For more information, call Kathy Glass, FRI at (608) 263-6935.

Oct. 9-10 North Central Cheese Industries Assn. Annual Convention. Minnesota. For information, call Dr. David Henning at (605) 688-5477.

Oct. 15-19 Wisconsin Cheese Technology Short Course. Madison, WI. Call Bill Wendorff at (608) 263-2015.

Oct. 18-19 Pasteurization Short Course for Trained Operators. River Falls, WI. Sponsored by UWRF. Program Coordinator: Ranee May (715) 425-3704.

Oct. 30-31 Membrane Processing of Dairy Products Short Course. Madison, WI. Program Coordinators: Bill Wendorff (608) 263-2015 and Karen Smith (608) 265-9605.

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