20, 19 turned out to be false positives and one dexamethasone-induced gene was identified; its full-length cDNA was cloned from a library and sequenced. The cDNA is a novel member of the Ras-superfamily and was named Dexras1 due to its ability to be induced by dexamethasone. Experiments are in progress to characterize the role of Dexras1 in the pituitary and in other steroid-responsive tissues. Overall, differential display was extremely useful in identification of a novel gene, however, it may require considerable effort in terms of testing various PCR primer combinations and the technique may be expected to generate a significant number of false positives.

**Key Words:** Differential , Display, Dexamethasone

**594 Genes, Chips and Animal Biology.** Nagappan Mathialagan*[1], Charles Bolten[1], Steven Wagner[1], John Byatt[1], and Frances Buonomo[1], [1]*Monsanto Animal Agricultural Group*.

Genomic technologies have transformed the animal biology research into a new era of discoveries in a similar fashion as the introduction of radioimmunoassay techniques. Genomics has resulted in the identification of thousands of new gene sequences in farm animal species with no real link to functional association. Comparative genomic analysis with completed genomes such as human has been used to discover orthologous genes. However, this approach still leaves out the annotation of genes which are novel to the animal species. Gene expression technologies like Microarrays and Serial Analysis of Gene Expression (SAGE) are used to determine the expression of thousands of genes simultaneously. Species-specific microarrays need to be used to associate a function to these new genes. However, cross-species microarrays may be used in instances where there are no species-specific arrays. We have used Incyte human microarrays for transcript profiling of bovine mammary gland to identify regulated genes associated with milk production. A set of human gene homologues were identified that are regulated during lactation and involution. Genes up-regulated during lactation, identified by heterologous profiling, were selected for confirmation by other methods such as Northern blot analysis, quantitative RT-PCR, subtractive cDNA libraries and nylon arrays. One example of a regulated gene we selected for confirmation was Stearoyl-CoA-Desaturase (SCD), an enzyme involved in the synthesis of conjugated linoleic acid. An increased expression was associated with lactation while a sharp decline in the expression was observed with involution. In addition, our experience with heterologous arrays showed that genes can be erroneously identified due to sequence identity of bovine genes to unrelated genes in human. This observation emphasizes the preference to use species-specific arrays for gene expression studies.

**Key Words:** Transcript Profiling, Microarrays, Genomics

**595 Proteomics in the animal sciences.** Lawrence Dangott*, *Texas A&M Universtiy, College Station, TX.*

One of the goals of biologists in the post-genome era will be to characterize all the proteins within an organism, tissue or organelle, in order to describe the pathways and protein interactions that mediate cellular function. Proteomics is the term given to the large-scale analysis of proteins using biochemical, biophysical and chemical techniques of analysis. Although traditionally associated with the two-dimensional display of large numbers of proteins, in the post-genomic era, proteomics is dividing into three main areas; 1) protein identification and microcharacterization; 2) differential expression analysis of proteins in normal and altered tissues; and 3) studying protein-protein interactions. Approaches to achieve these goals require the combination of traditional molecular biological, biochemical and biophysical techniques with the expanding capabilities of high-throughput robotics and high-sensitivity, high-resolution mass spectrometry as well as the development of new technologies. These kinds of approaches are being used in our laboratory and others to explore and explain the functions, interactions and regulation of proteins in animal reproductive biology and environmental toxicology. Proteins involved in embryo implantation are being identified in ovine uteri using ‘knock-out’ ewes, two-dimensional gels and in gel digestion techniques coupled to automated protein micro-sequencing and MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry. Similarly, proteins involved in horse sperm differentiation are being identified by applying these techniques to proteins isolated from in vitro cultures of equine seminal tubules. In related experiments, post-translational modifications are being mapped by ion-trap electrospray mass spectrometry and multi-dimensional chromatography coupled with mass spectrometry is being used to identify components of protein complexes.

**Key Words:** Proteomics, Mass spectrometry

## Preharvest and Postharvest Approaches to Modification of Milkfat

**596 The milk fat globule membrane of buttermilk: a unique ingredient.** M. Corredig*, *Department of Food Science and Technology, University of Georgia.*

The presence of material derived from the milk fat globule membrane (MFGM) makes buttermilk (the byproduct of buttermaking) distinct from any other dairy product. Studies of MFGM have revealed strong associations of the membrane lipids with various membrane proteins (butyrophilin, xanthine oxidase and some minor proteins). When membrane material is isolated from buttermilk a high ratio of polar lipids is found, in particular phosphatidyl ethanolamine, phosphatidyl choline and sphingomyelin. Phospholipids play an important role in many metabolic processes, and phospholipid-enriched fractions are today marketed as important ingredients in a variety of dietary products. Furthermore, evidence is emerging that sphingomyelin from milk may have anti-cancer properties and other health-related benefits. In addition to the nutritional quality of MFGM, a more detailed analysis of the composition of buttermilk has suggested the utilization of buttermilk as an ingredient in the manufacture of foods, for example low fat cheese and yogurt. The behavior of buttermilk as a functional ingredient can be attributed to the presence of skim milk proteins and the MFGM, however the role played by the various components and their interactions is not understood. Processing history and compositional differences also seem to affect the functionality of MFGM. Understanding the various components and the functional properties of buttermilk will allow this byproduct to become a source of new and unique ingredients. Our discussion will review the current literature in this area and present some thoughts on the further development of commercial products derived from the MFGM.

**Key Words:** phospholipids, MFGM, buttermilk

## Role of Extracellular Matrix (ECM) in Growth and Development

**597 The role of the extracellular matrix in growth and development: An introduction.** M.W. Orth*, *Michigan State University.*

Besides providing structural support, the extracellular matrix (ECM) has recently been shown to play a significant role in the regulation of tissue growth and development. As an example, certain ECM molecules can sequester growth factors and release them during tissue remodeling. Also, proteolytic products of ECM molecules can have a unique biological activity via interactions with cell surface receptors. The focus of this symposium will be to examine the role and regulation of the ECM in four tissues of particular interest to the animal scientist. Dr. Sandy Velleman will describe the architecture of the ECM in skeletal muscle tissue with an emphasis on the function of the proteoglycan component. The predominant focus on myofibrillar proteins traditionally has overshadowed this exciting area of research. Dr. Tom Schmid will discuss the role of the ECM during endochondral bone formation, with an emphasis on ECM proteins found in unique regions of growth plate and articular cartilage that were discovered in his laboratory. He will also discuss the potential of using ECM molecules as biological markers in physiological fluids to monitor the development and health of the skeleton. Dr. Russ Hovey will discuss the development of the mammary gland and its complex architectural structure. Specifically his research interest focuses on understanding the contribution of the mammary stroma dur-

ing mammary epithelial proliferation and function. Finally, Dr. George Smith will elaborate on his research into the role of matrix metalloproteinases and plasminogen activators in ovarian function. His laboratory has found that these two families of ECM proteinases are temporally and spatially regulated during the ovarian cycle and contribute to ECM remodeling associated with growth, differentiation, and resorption of ovarian structures. We hope that through this symposium animal scientists will gain a deeper appreciation for the ECM and how it could play a major role in understanding and solving some of the problems encountered in the animal science industry.

**Key Words:** growth, development, matrix

### 598 Role of the extracellular matrix in muscle growth and development.

Sandra G. Velleman*[1], [1]*The Ohio State University/OARDC.*

Studies on the growth and development of skeletal muscle have predominantly focused on the myofibrillar components especially myosin and actin. It has been largely overlooked that skeletal muscle cells secrete a network of extracellular matrix macromolecules which have the potential to regulate the growth and development process. The extracellular matrix is composed of a network of glycoproteins, collagen, and proteoglycans that surround the skeletal muscle fibers. The positioning of the individual extracellular matrix macromolecules is not random, but instead the individual components interact to form a precise structural architecture which becomes the epimysium, perimysium, and endomysium skeletal muscle connective tissue layers. The extracellular matrix then functions by maintaining tissue shape and structure, regulating tissue function, and communicating information back to the cells through the formation of cell-extracellular matrix signal transduction pathways. Information communicated to the cell from the extracellular matrix may influence migration and adhesion, and proliferation and differentiation. As muscle ages, the composition of the extracellular matrix changes. During muscle formation, the embryonic extracellular matrix appears to be involved in the spacing of the developing muscle fibers and later in development changes to a matrix with macromolecular components involved in growth factor regulation. Although basic aspects of skeletal muscle extracellular matrix composition are known, it is not well understood how the extracellular matrix affects skeletal muscle development and function. Focus will be on the proteoglycan component of the extracellular matrix because of its role in regulating collagen fibrillogenesis, cellular growth properties, and cellular growth factor responsiveness.

**Key Words:** Muscle, Proteoglycans, Extracellular Matrix

### 599 Role of the extracellular matrix in skeletal growth, development and health.

T. M. Schmid*, *Rush Medical College, Chicago, IL.*

The mass and volume occupied by the skeleton is primarily due to its abundant extracellular matrix (ECM). Research over the last 3 decades has characterized many skeletal macromolecules including classes of collagens, proteoglycans and glycoproteins. These molecules fulfill different structural functions, but also can exhibit regulatory roles. The ECM provides a scaffold for skeletal remodeling and acts as a reservoir for growth factors and nutrients. The matrix of cartilage and bone are distinctly different, however their calcified cartilage interface is surprisingly similar. The growth of this organ is orchestrated by a symphony of growth factors. The fine-tuning of this metabolic activity requires an information exchange at the cell-matrix interface. Cell surface receptors bind extracellular matrix ligands and generate intracellular signals, which direct cellular activities. Fragments of matrix macromolecules can modulate these interactions. The remodeling of the extracellular matrix in growth and development is a highly regulated process involving several classes of proteolytic enzymes. A variety of biochemical assays have been developed to study the synthesis and degradation of the extracellular matrix in cartilage and bone. Work in my laboratory has focused on the identification of macromolecules that are unique to specific regions of growth or articular cartilage. Type X collagen is the prototype of these molecules and is found almost exclusively at the interface of cartilage and bone. Another exciting molecule, which protects the articular cartilage surface, is superficial zone protein (SZP). This is a 345-kDa proteoglycan that is synthesized by superficial zone chondrocytes, but not deep zone chondrocytes. We have recently shown this molecule to be an abundant glycoprotein in synovial fluid and has lubrication activity when tested on a cartilage-glass interface. Another protein Del1 appears to be enriched in the superficial layer of articular cartilage and in the hypertrophic zone of the growth plate. Future studies will define if these molecules hold promise as biochemical markers to monitor the growth, development and health of the skeleton.

**Key Words:** Growth plate, Cartilage, Skeleton

### 600 Role of the stroma and extracellular matrix during mammary gland growth and development.

R.C. Hovey*, [1]*National Cancer Institute, NIH.*

The normal mammary gland undergoes extensive development prior to assuming the ultimate lactogenic phenotype. Under hormonal regulation the mammary epithelium proliferates within the stromal matrix of the mammary fat pad, giving rise to an architecturally-complex and species-unique ductal network. These species differences reflect an altered abundance and proliferation of various stromal cell types (adipocytes, fibroblasts, endothelial cells) that intimately associate with myoepithelial and epithelial cells. Together these cells contribute the various elements of the extracellular matrix that regulate normal epithelial cell proliferation, differentiation and milk protein gene expression, and tumorigenesis and metastasis during breast disease. Historically, attention has focused on the function of mammary epithelial cells, independent of the stromal matrix. Our research has begun to explore the contribution of the mammary stroma during mammary epithelial proliferation and function. It is increasingly evident that cellular constituents within the mammary fat pad are integral sites of hormone action that mediate systemic signals through altered stromal proliferation and paracrine growth factor expression. Likewise, species differences in stromal function may confer differential morphogenesis to the mammary epithelium. In addition, development of the vasculature system within the mammary gland occurs within the mammary fat pad under hormonal and growth factor influences that converge during epithelial-stromal and extracellular matrix interactions. Although the mechanisms by which epithelial-stromal and epithelial-matrix-stromal interactions regulate mammary gland growth, morphogenesis, function and neoplasia have not been resolved, increasing evidence points to their integral function within the mammary gland.

**Key Words:** mammary, stroma, epithelial-stromal interaction

### 601 Regulation of extracellular matrix remodeling during the ovarian cycle: Implications for the control of growth, differentiation and resorption of specific ovarian structures.

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For many years, the ovarian extracellular matrix (ECM) was thought to function merely as scaffolding providing architectural support. However, a growing body of evidence indicates the ovarian ECM also plays a key regulatory role. In general, changes in the ECM can influence gene expression and cell migration, proliferation, differentiation and death. Within the ovary, changes in the ECM help regulate specific events during the ovarian cycle. Growth of bovine ovarian follicles from the primordial to preovulatory stage is characterized by an approximately 360,000-fold increase in surface area as follicles expand within the confines of the surrounding ovarian stroma (ECM). Ultimately, most follicles die by atresia and are resorbed. The remaining follicles undergo ovulation, characterized by follicle rupture and release of the egg. Follicle rupture is dependent upon localized degradation of the ECM at the apex of the preovulatory follicle wall. Extensive ECM remodeling is also characteristic of the luteal phase. Following ovulation, transformation of remnants of the preovulatory follicle into a corpus luteum involves mechanisms similar to wound healing and tumor formation. Disruption of cell-ECM contacts, loss of steroidogenic capacity, cell death and tissue resorption characterizes corpus luteum regression at the end of a nonfertile cycle. Two families of proteinases that regulate ovarian ECM remodeling are the matrix metalloproteinases (MMP) and the plasminogen activators (PA). The MMP are a large gene family that digest various ECM components and are noted for their role in ECM remodeling during tissue growth, morphogenesis and repair. The PA are implicated in control of ECM remodeling via their ability to convert the ubiquitous zymogen plasminogen to its active form plasmin, which directly and indirectly mediates ovarian ECM degradation. The fundamental

role of the ECM in the control of growth, differentiation, and resorption of ovarian structures and the regulation of ovarian ECM remodeling by the MMP and PA will be discussed. Our results indicate that the MMP and PA are temporally and spatially regulated during specific stages of the ovarian cycle and control ECM remodeling fundamental to ovarian function. Supported by USDA 98-35203-6226 (GWS) & 98-35203-6282 (MFS).

**Key Words:** Ovarian Extracellular Matrix Remodeling, Matrix Metalloproteinases, Plasminogen Activators

## ADSA Dairy Foods: Cheese

### 602 Quality attributes of cheddar cheese in the North Carolina marketplac.

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Approximately 250 samples of cheddar cheese were obtained from various retail markets in North Carolina. Cheddar cheese samples were evaluated over a two year period. The evaluation was conducted with 15 trained dairy judges. Attributes were appearance, body and texture and they were evaluated according ADSA protocol. The major defects in appearance were open and a few samples were gassy. The major body defects were, from most to least, short, crumbly, pasty and curdy. The flavor defects from most to least were high acid, bitter, whey, sulfide, unclean, heated, flat and yeasty. The national brands of cheeses had less appearance and body defects. The main flavor defects were high acid, bitter, sulfide and whey, whereas the store brands tended to be more open and gassy in appearance. The body and texture would be short, curdy, crumbly and pasty depending on the brand. The store brands tended to have more off flavors such as unclean, oxidized, yeasty, flat, whey, sulfide, fermented and fruity. The store brands of sharp cheese tended to be acid, bitter and sulfide as compared to the national brand cheese which were acid and sulfide. The national brands usually had a cleaner flavor and were better qualit

**Key Words:** Cheddar Cheese, Marketplace, Quality

### 603 Salt and calcium distribution in injected cheese.

A.J. Pastorino*[1], N.P. Ricks[2], C.L. Hansen[1], and D.J. McMahon[1], [1]*Utah State University,* [2]*Ohio State University.*

Modifying cheese attributes by injecting ionic solutions requires knowledge of the time needed to obtain uniform concentration of ions in the cheese. Therefore, our objective was to determine whether even distribution of sodium chloride (salt) and calcium chloride in cheese, over 1.0 cm distance from injection site could be obtained after 30 to 45 d. Full-fat salted cheese (25% fat, 45% moisture) and reduced-fat unsalted cheeses (22% fat, 42% moisture) were made according to direct-acid, stirred-pressed curd procedures. Cheeses were cut into 0.4 to 0.6-kg blocks, vacuum-packaged, and stored at 4°C. After 3 wk of storage, full-fat cheese blocks were high-pressure injected with a 40% calcium chloride solution, while reduced-fat blocks were injected with a 23% salt solution. Injection was performed in a single, centered row, in the top of the cheese block, with injection sites 1 cm apart. Pressure of injection was set at 1500 psi, and burst duration at 1.5 s. Cheese blocks were then vacuum-packaged and stored at 4°C. After storage, cheeses were sectioned into bands, 0.5 cm thick, parallel to the injection line. Three bands to each side of the injection line were considered for analysis, and the chloride and calcium content determined. Salt-injected cheese was analyzed 6 wk after injection and had increased salt content compared to uninjected cheese, 0.25% versus 0.16% ($P < 0.01$). Also, the injected cheese had an even distribution of salt over 1.5 cm from the injection line. Calcium-injected cheese, analyzed 4 wk after injection, had increased calcium content when compared to uninjected cheese, 0.43% versus 0.33% ($P < 0.01$), and had even distribution of calcium over 1.0 cm from the injection line. Upon injection, increased localized ion concentration generated a concentration gradient in the cheese that operated as a driving force for ions to diffuse. Also, deflection of the injectant as it enters the cheese would provide some initial dispersion of the solution. We concluded that injecting concentrated solutions of sodium and calcium chloride allows for increasing their content in the cheese, and that injecting these solutions using a 1 x 1-cm injection pattern allows for even distribution of ions in 30 d.

**Key Words:** Cheese, Salt, Calcium

### 604 Characterization of the melt properties of Cheddar cheese during ageing using dynamic low amplitude oscillatory rheology and melt profile analysis.

Achyuth Hassan* and John Lucey, *University of Wisconsin-Madison.*

Melt characteristics are an important functionality of cheese on pizza. The melting properties of Cheddar cheese during ageing were determined by melt profile analysis and two dynamic low amplitude oscillation (DLAO) tests on a Physica UDS 200 rheometer. The newly developed melt profile method measures changes in cheese height during heating from 12 to 60$^o$C and provides information on extent of flow and softening temperature. Extent of flow values (i.e., decrease in original cheese height) for 1, 7, 14, 21, 30, and 90 d samples were 54, 61, 64, 71, 71.5, and 71.5%, respectively. Rheological properties of cheese were evaluated at a strain of 0.2% and frequency of 0.1 Hz. Storage modulus (stiffness) and loss tangent parameters were determined from DLAO tests during heating. Cheeses were given two different types of heating profiles; one profile was the same as that used in the melt profile analysis technique (a non-linear but short heating profile or SHP). In the second heating profile, cheeses were heated from 5 to 80$^o$C at constant rate of 1$^o$C/min (long heating profile or LHP). In both heating profiles the loss tangent remained constant up to 40$^o$C ( 0.5) and thereafter increased. The temperature when the loss tangent initially increased was similar to the softening temperature determined by melt profile analysis. Loss tangent values (at temperatures >40$^o$C) increased with the age of cheese. In SHP tests, the values of the loss tangent at 55$^o$C were 0.7, 1.3, 1.5, 1.7, 2.0, and 2.3 for 1, 7, 14, 21, 30, and 90 d cheese, respectively. In samples subjected to LHP, a well defined peak was observed in loss tangent and maximum values were 1.2, 2.0, 2.3, 2.5, and 2.1 which occurred at temperatures of 69, 67, 65, 64, 62, and 57$^o$C for 1, 7, 14, 21, 30, and 90 d cheese, respectively. The decrease in height of cheese samples during heating paralleled trends in the increase in loss tangent value from rheological tests. An increased loss tangent indicates a change in character of cheese from solid-like material to viscous or liquid-like and it appears that this increase is involved in melting and flow of cheese at high temperatures. With increasing age and proteolysis the extent of flow increased and this coincided with higher loss tangent values in rheological tests.

**Key Words:** Rheology, Cheese, Melt profile

### 605 Reduced fat Cheddar cheese from a mixture of cream and liquid milk protein concentrate.

Shakeel Rehman* and Nana Farkye, *Dairy Products Technology Center, Calpoly State University.*

Liquid milk protein concentrate (LMPC) is a high protein and low lactose dairy ingredient manufactured by ultrafiltration of skim milk. The study was undertaken to use LMPC and cream mixture in reduced fat Cheddar cheese (RFC) manufacture in order to increase yields. Control RFC was manufactured from standardized milk casein/fat, C/F 1.7, obtained from mixing whole milk (WM) and skim milk (SM) while experimental RFC was manufactured from standardized milk, C/F 2.0, obtained from mixing LMPC and 35 % fat cream. The % yield, % total solid (TS) and fat recoveries in the experimental RFC were 21.2, 61.4 and 85.4 as compared to 8.0, 45.1 and 77.3 in the control RFC, respectively. The average % moisture, fat, protein, salt and lactose were 40.7, 15.3, 32.8, 1.4 and 0.07, respectively, in the experimental cheese and 39.3, 15.4, 33.0, 1.3 and 0.10, respectively, in the control cheese. No growth of non-starter lactic acid bacteria (NSLAB) was detected in control or the experimental cheeses up to 3 mo ripening but at the end of 6 mo ripening the experimental cheese had $10^7$ cfu NSLAB / g as compared to $10^6$ cfu / g in the control. The experimental cheese had lower levels of water soluble N (as % of total N) than the control cheese after 6 mo ripening, suggesting lower levels of primary proteolysis in the experimental cheese. The total free amino acids, determined by Cd-ninhydrin method, were significantly lower in the experimental cheese than the control cheese during 6 mo ripening, suggesting lower