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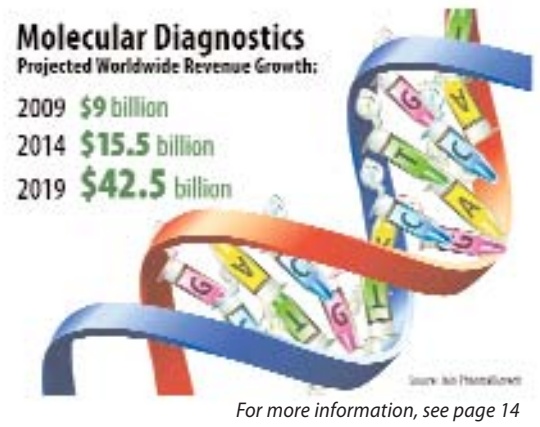
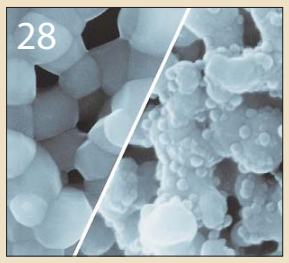


For more information, see page 14



Hydroxyapatite for Biomolecule Purification

HA usage has grown in popularity, and it is now a mainstream industrial tool.

Aseptic Filling Adjusting to New Paradigm

Rise in drugs' complexity and sensitivity presents unique production challenges.

Mass Spec Central to Metabolomics

Range of Methodologies Are Being Deployed to Advance New Drug Development

K. John Morrow Jr., Ph.D.

The emerging field of metabolomics would be hobbled by irresolvable technical challenges were it not for the expanding availability of user-friendly and affordable mass spec instruments. A German Research Center for Environmental Health conference, "Metabolomics & More—the Impact of Metabolomics on the Life Sciences," held last month in Munich, showcased a range of initiatives under way in commercial and academic labs that harness the power of mass spectrometry to measure and monitor the metabolome.

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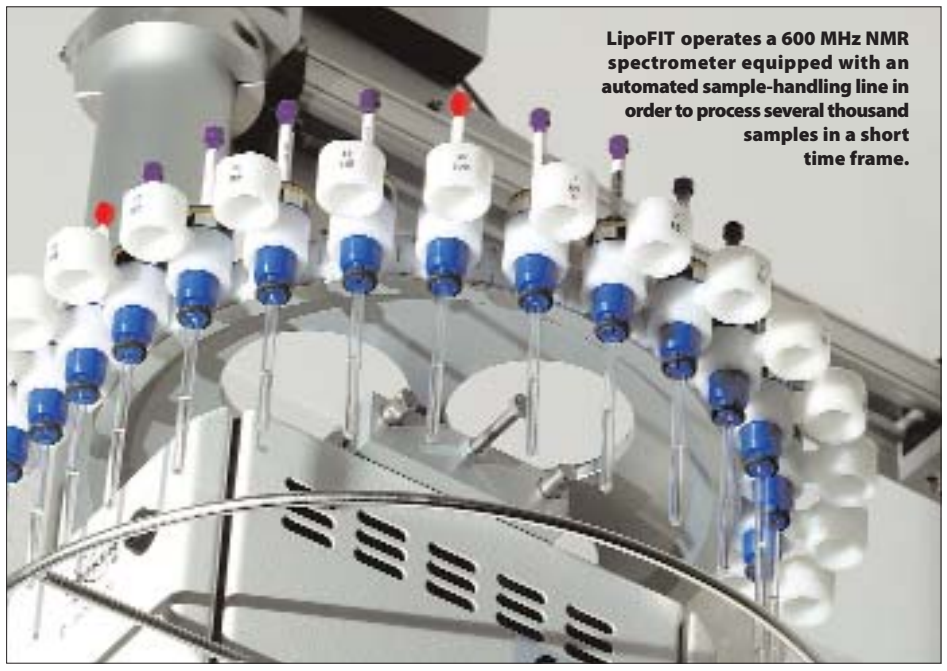
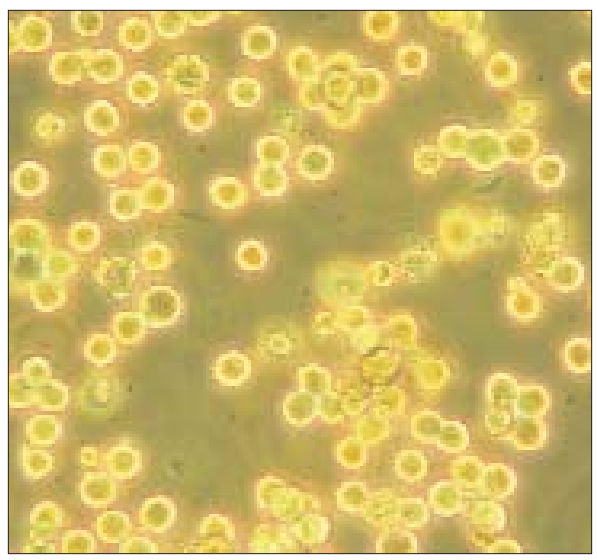


LipoFIT operates a 600 MHz NMR spectrometer equipped with an automated sample-handling line in order to process several thousand samples in a short time frame.

Cell Culture Moving Past Previous Technical Limits

Researchers Employ Insect and Duck Cells, Novel Media, and Next-Generation Automation Technologies



CHO-K1 cells adapted to growth in a protein- and peptide-free minimal medium developed by Cell Culture Technologies: the cells proliferate in minimal media either under static or agitated culture conditions.

Gail Dutton

Spodoptera frugiperda (the fall armyworm caterpillar) and the duck are gradually replacing the chicken as the creature of choice for vaccine production. New cell-culture techniques and, particularly, recombinant technologies, are bringing faster production cycles and higher purity yields, according to researchers who will present their insights at the Bharatbook conference on "Vaccine Manufacturing" next month in London.

Protein Sciences (www.proteinsciences.com) is about to commercialize its FluBlok™ seasonal flu vaccine, which was developed through a baculovirus protein-expression technology that uses insect cells as host cells. The *Spodoptera frugiperda* insect cell line (expresSF+® cells) grows in suspension culture in the absence of serum. "We will have one of the first products made in insect cells licensed in the U.S.," reports Clifton McPherson, Ph.D., director of quality control.

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Sticky ends

Affymetrix released GeneAtlas™ personal microarray system for whole-genome gene expression... **BioStorage Technologies** launched Osiris™ mobile biorepository... **Ambry Genetics** and **RainDance Technologies** are partnering to offer diagnostic and genomic services utilizing the latter's RDT 1000 sequence enrichment solution... **Corning** introduced Synthemax™ synthetic surface that reportedly supports growth and differentiation of stem cells... **Sigma Life Science** extended its portfolio of knockout rats with new suite of models for ADME/Toxstudies... **Ricerca Biosciences** finalized acquisition of the discovery and preclinical business of **MDS Pharma Services**... **Harvard Bioscience** is marketing the Organizer™ series model 100 In Breath bioreactor, which was used to grow the bronchus for the clinical transplantation performed by Dr. Paolo Macchiarini of Barcelona-based Hospital Clinic... AnaSpec, the newest North American division of **Eurogentec**, now offers custom oligonucleotide synthesis.

Mary Ann Liebert, Inc. publishers

Metabolomics

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Klaus Weinberger, Ph.D., CSO of **Biocrates** (www.biocrates.com), is a fan of targeted metabolomics because it allows his company to "scale-down analytical challenges and produce an accurate reflection of environmental influences on the individual subject." Dr. Weinberger discussed Biocrates' progress in uncovering new biomarkers of metabolic conditions at the meeting. "We focus on a number of current issues, including diseases with long latent phases, complex health conditions such as diabetes, and improvement of current diagnostics."

There is a paradigm shift afoot in the field of clinical diagnostics, in which high-resolution biochemical characterization of body fluids by targeted metabolomics is being counted on to facilitate the development of function-oriented diagnostics, moving from expensive immunoassays to cheaper, more specific, and more accurate mass spec assays. These new diagnostics are much more than just a positive or negative test for the condition, they also allow detailed subtyping and staging, which will direct personalized therapeutic regimens, Dr. Weinberger said.

Biocrates is concerned with a range of diagnostic markers, including screening markers for early diagnosis, prognostic biomarkers that measure disease progression, theranostic markers for optimizing drug protocols, and markers of inborn metabolic diseases for newborn screening.

The concept of inborn errors of metabolism harkens back over a century when it was first formulated by Sir Archibald Garrod, who recognized that certain diseases in newborns were the result of gene defects causing metabolites to pile up in their serum and urine. Diseases such as phenylketonuria have long been part of the neonatal testing repertoire, but the traditional methods tested for only one possible genetic disorder at a time.

It is now possible to screen newborns for a whole range of conditions, instead of using laborious and expensive one-shot assays, Dr. Weinberger said, adding that Biocrates has developed a screen for 20 to 30 single gene diseases.

Studies at Biocrates have demonstrated that mass spec-based diagnostics can provide much greater coverage, while at the same time, covering a greatly expanded range of diseases, Dr. Weinberger explained. Specifically, quantitative tandem mass spec with stable isotope dilution will meet stringent quality criteria. Depending on the needs of the diagnosis, researchers can use either a targeted metabolomics approach or metabolic profiling with a LC/MS full scan.

Biocrates' integrated technology platform combines sample preparation, analytics, and bioinformatics, which permits the processing of large numbers of samples. "Our platform can be applied to a wealth of areas, such as basic investigations, agricultural research, drug discovery, and clinical diagnostics and therapeutics."

Metabolomes Under Stress

"Measuring and monitoring the metabolome are perhaps two of the most daunting challenges of the 21st century," according to Herbert Hill, Ph.D., professor at Washington State University. Our current level of ignorance is illustrated by the fact that estimates for the number of metabolites in the human and plant metabolomes range from around 2,800 to well over 200,000. And these compounds run the gamut from polar to nonpolar, with examples of every conceivable functional group. Indeed, some classifications are so weighty that they require their own subclassification such as the lipodome and the glycome.

Chirality and a profusion of isomers add to the challenge. While nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy have been utilized for analysis of the metabolome, both approaches have their limitations. For this reason, Dr. Hill and his collaborators have focused on ion mobility mass spec (IMS) to analyze bodily fluids.

"When coupled with mass spec, ion mobility spectrometry offers value-added data not possible from mass spec alone," Dr. Hill explained. "Indeed, the two methods complement and match one another like a hand in glove and effectively become one analytical instrument."

Dr. Hill's team has confirmed the power of this technology through metabolomic studies of lymphatic fluid from rats monitored while under dietary stress. Nutri-

tional deprivation as well as diabetes and obesity may cause various metabolic changes, which, in turn, could lead to cancer and cardiovascular disease, so the ability to closely monitor the metabolome could be a critical tool in understanding and eventually treating these conditions.

Drug Mechanism Studies

The comprehensive study of metabolic reactions is producing a large amount of information, as global biochemical profiling is becoming the hot technology.

"We are employing metabolomics investigations to clarify the mechanism of action of several new compounds," said John Ryals, Ph.D., CEO at **Metabolon** (www.metabolon.com). The company is using its metabolomics platform technology in a range of studies, including following the global effects of disease processes on metabolism in order to uncover new biomarkers.

At the meeting, Dr. Ryals discussed studies with the putative anticancer compound GMX1777/1778, a member of a class of molecules known as cyanoguanidinopyrimidines that are thought to be involved in the inhibition of NF-kB.

The approach followed by Dr. Ryals and his collaborators is essentially similar to that of the Hill group, in which cells are exposed to the agent and the changes in their metabolome followed over time. The most dramatic change in the global profile of the cells was an increase in the levels of glutamine and a-glutamylglutamine and a decrease in the levels of nicotinamide and NAD+.

Cell rescue experiments demonstrated that nicotinic acid and nicotinamide will save the cells from the killing effects of the anticancer agent. These observations are reminiscent of studies on cancer cells of almost a century, in which Otto Warburg demonstrated the heavy reliance of tumor cells on glycolysis for ATP generation, driven by NAD+.

These straightforward investigations point the way toward the understanding of the mechanism of action of GMX1777/1778, demonstrating that the metabolic intermediate NamPRT is the primary target and not NF-kB.

In an unrelated project, Metabolon looked for a biomarker that would complement PSA, a protein long used for detection of prostate cancer. Screening a large number of metabolites in normal, benign, and malignant cells, they found sarcosine to be a marker for aggressive prostate cancer.

Glycine is converted to sarcosine by S-adenosyl-methionine transfer as a result of methylation scavenging, and hypermethylation appears to be correlated with tumor aggressivity. Preliminary investigations indicate that sarcosine may be a useful marker of prostate cancer invasion and aggressivity. If so, sarcosine may have potential both as a biomarker and as a therapeutic target.

A third project aims to identify biomarkers for insulin resistance, aiming at validation of a compound that can predict the level of insulin resistance in nondiabetic at-risk patients. One marker, alpha hydroxybutyrate is better than adiponectin at correlating to insulin resistance, Dr. Ryals said. To validate this marker, the team followed individuals who underwent dramatic weight loss following bariatric surgery. "Alpha hydroxylbutyrate was the most significantly altered metabolite at one year post-bariatric surgery."

"We have developed a high-throughput screening assay that allows us to detect small molecules with tumor-suppressing activity," explained Gerhard Wagner, Ph.D., professor at Harvard Medical School. Dr. Wagner and his associates have studied the protein complex elF4E/elF4G, which is required for elongation of protein molecules on the ribosome. Misregulation of this complex is responsible for a variety of ills, including tumorigenesis and other forms of aberrant cell growth. For this reason the complex is a tantalizing target for small molecules that act as inhibitors.

Dr. Wagner and his co-workers devised a high-throughput fluorescence polarization assay that was validated with peptides designed for the task. This tool was applied to the massive screening of 16,000 compounds in the Cambridge DiverSet E Library, from which a high-binding candi-

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Metabolomics

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date, christened 4EGl-1, was retrieved. This material disrupts the ElF4F complex, bringing translation to a grinding halt.

"Our results show that we can inhibit the interaction between the cap binding proteins with small molecules identified through our library screening," Dr. Wagner stated. "It is likely that inhibition of cap-dependent translation will thus have therapeutic value against a wide range of cancer types."

LipoFIT (www.lipofit.de) offers a range of services based on nuclear magnetic resonance tomography, according to Werner Krämer, Ph.D., co-founder and technical director. NMR can be adapted to the determination of the molecular structure of proteins, glycoproteins, nucleic acids, and many other classes of molecules. Because it does not destroy or damage the sample, it does not suffer from artifacts common to many other analytical procedures.

NMR tomography can be applied to agricultural, as well as medical challenges. The precision of the technology allows analogues and other minor contaminates that might cause severe immune reactions or other serious side effects to be detected and the responsible bad actors eliminated.

PPARs

The peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear receptor superfamily. Metabolomic studies form a tool for studying their role in obesity, diabetes, and carcinogenesis, reported Jules Griffin, Ph.D., of Cambridge University.

Dr. Griffin's lab has focused its efforts on understanding the role of these different receptors in response to abnormally rich diets, which are positively correlated with all these conditions. For instance, the receptor known as PPARa, which is especially high in metabolically active tissues such as liver, kidney, heart, skeletal muscle, and brown fat is involved in fatty acid oxidation.

"Our metabolomics investigations have highlighted a number of perturbations affecting systemic metabolism in the PPARa knock-out mouse," Dr. Griffin explained. "These include progressive hepatic steatosis, reductions in the concentrations of glucose and glycogen in both liver and muscle tissue, and reduced b-oxidation (known targets of PPARa receptor)." Following this line of investigation, metabolic phenotypes can be used to artificially age the animal and investigate the interaction between age and metabolic diseases.

Diagnostic Tool

The "Tricorder," a mysterious diagnostic tool prominently featured on "Star Trek," may be moving closer to reality, according to Teresa Fan, Ph.D., director of the center for regulatory and environmental metabolomics at the University of Louisville.

The current prototype is a two-story-high 800 MHz NMR spectrometer, with its attendant liquid nitrogen tanks. Dr. Fan and her co-workers are investigating metabolic dysregulation using stable isotope resolved metabolomics. Specifically, the studies involve the element selenium and its role in cancer prevention. The chemical form of selenium has been shown to have an anticancer effect.

Progress in NMR and mass spec make complex metabolomic characterizations possible. Molecules can be labeled with heavy isotopes and their progress can be traced in selenium-treated and control materials. Dr. Fan's research has revealed that the anticancer properties of selenium are a result of the element's effect on the disrupted Kreb's cycle metabolism of the cancer cells.

These observations play into the concept of a noninvasive diagnostic instrument. "Our current understanding of the distinctive biochemical phenotype of lung cancer focuses on accelerated glycolysis and enhanced glucose uptake," Dr. Fan said. In the future, metabolomic studies based on heavy isotope labeling could lead to early, highly sensitive diagnostics, avoiding the costly false positives that characterize current diagnostic tools.

In the last two decades many targeted approaches to discovery have been proposed, explored, and found wanting. Now a new contender is vying for attention, and preliminary results hold promise. As studies unfold, the promise of metabolomics as a refined tool for eliminating the weed patch of dead-end drug candidates will be vindicated or denied. GEN