

Division of Mass Spectrometry

DIVISION PROFILE

Number of Faculty	1
Number of Fellows	
Research Fellows	1
Number of Support Personnel	4
Annual Total Industry Contracts (direct)	\$192,500
Number of Peer Reviewed Publications	15

FACULTY LISTING

Kenneth D.R. Setchell, PhD, Professor of Pediatrics, Director, Clinical Mass Spectrometry

OVERVIEW

The Clinical Mass Spectrometry Laboratory provides an analytical resource focusing on the application of mass spectrometry and allied chromatographies to the analysis of small molecules, generally of less than 1000 daltons molecular weight, in complex clinical and biological samples. The goals of the program are to better understand the biochemical basis of disease, to improve diagnostic approaches to detection of early-stage disease and to explore mechanisms that lead to improved treatments or prevention. The facility, located on the R-Level of the Research Building, is equipped with a high resolution VG Autospec Q GC-MS-MS instrument, and a Micromass LC Quattro Electro spray Triple quadrupole instrument. These instruments are complemented with 4 capillary column gas chromatographs and 3 high pressure liquid chromatography (HPLC) systems having ultraviolet, fluorescence and electrochemical detectors which are available for method development and analytical support.



K. Setchell

This inventory affords a powerful analytical resource.

The division has an active independent research program applying mass spectrometry to the diagnosis and treatment of many diseases, and to the understanding of developmental biochemistry, physiology and pathophysiology. The technique of mass spectrometry provides detailed structural information based upon the molecular weight and fragmentation of a molecule, offers extremely high sensitivity and precision for accurate quantification. Since it is possible to distinguish stable-isotopically labeled analogs by their differences in molecular weights, mass spectrometry is a powerful tool for stable-isotope tracer and kinetic studies in infants and children, circumventing the use of radioactive tracers. The facility is recognized as one of the leaders in the area of cholesterol, steroid, phytoestrogen, and bile acid metabolism and consequently has built a strong national and international program in these areas of research. Training in analytical biochemistry is available to clinical fellows and graduates, and a strong emphasis on method development has led to collaborations with investigators from other departments within the Institute and outside.

Utilizing electrospray mass spectrometry routine methods for a number of important drug assays, including the immunosuppressants, Sirolimus and Tacrolimus, and the anticonvulsant drug, topiramate, have been developed and are in clinical use. Research studies using these methodologies have included clinical drug monitoring and understanding of pharmacokinetics of these drugs, that is especially important in pediatrics because it cannot be assumed that the metabolism and efficacy of drugs is the same in adults and children. These recent developments are aimed at placing the Mass Spectrometry at the center of a program aimed at establishing a Core resource for studies on pharmacokinetics and clinical monitoring of these drugs.

HIGHLIGHTS

The independent research program continues to focus in two major areas but the program has widened recently to include a greater emphasis on clinical pharmacology. Collaborations have been established with the Division of Laboratory Medicine (Dr. Paul Steele) and Clinical Trials Office (Dr. Sander Vinks) and the Division of Neurology (Dr. Tracy Glauser). There continues a strong program of research on the role of nutrition, specifically focused on studies investigating the role of phytoestrogens in disease prevention and treatment. Phytoestrogens are hormone-like natural compounds that have been found to act as selective estrogen receptor modulators, having the ability to confer many of the benefits of estrogens without the negative effects of estrogens. These compounds are found in high levels in soybeans, soy foods and flax, and diets containing phytoestrogens are widely under investigation for their potential for disease prevention in areas of breast and prostate cancer, cardiovascular disease and osteoporosis. The Mass Spectrometry Division has developed methodologies for analysis of phytoestrogens and is playing a prominent role in collaborations with many international research groups that are studying the health benefit of these natural bioactive plant constituents. A key intestinally derived metabolite of soy isoflavones, called equol, is extensively under investigation because it has been found to be more biologically active than the precursors from which it is synthesized. Equol exists in two diastereoisomers and we have shown that intestinal bacteria produce exclusively the S-equol enantiomer. S-equol and R-equol differ significantly in their binding affinity for estrogen receptors. In collaboration with Brigham Young University and Colorado State University we have found that both enantiomers are potent antagonists of dihydrotestosterone, suggesting that equol may have important effects in treating or preventing androgen-mediated diseases or conditions. This work has led to the filing of several Patents on the application equol and we have been working with the Office of Intellectual Property and Venture Development with the view to advance the technology further.

The synthesis and metabolism of cholesterol and bile acids as these pertain to liver and gastrointestinal diseases remains a major area of research. In the course of applying mass spectrometry at CHMC we have discovered a total of 6 genetic defects in bile acid synthesis, and provide an international clinical service for the screening, diagnosis, and treatment of infants and children with liver disease caused by these inborn errors in bile acid metabolism. We have developed a treatment strategy involving bile acid therapy that leads to complete reversal of liver injury circumventing the need for liver transplantation that otherwise would be required for most of these patients with advancing disease. Therapy is conducted under an Investigational New Drug (IND) approval from the FDA but in view of its demonstrable safety and efficacy we have now applied for Orphan designation status for cholic acid therapy in specific inborn errors.

Completion of 'proof-of-principle' studies in the area of colon cancer prevention has resulted in the demonstration of significant anticancer effects of the disulfate conjugate of ursodeoxycholate (SUDCA). These studies formed part of the licensing agreement with Axcan Pharma in Montreal, Canada, for our patents US patent 5,763,435 ("Sulfate conjugates of ursodeoxycholic and their beneficial use in inflammatory disorders and other applications") and US Patent 6,251,884 ("Sulfate conjugates of ursodeoxycholic acid and their beneficial use in inflammatory disorders and other applications"). Using a classical animal model for colon cancer, the rat model of azoxymethane-induced colon cancer, we have shown that SUDCA has powerful chemopreventive actions in the colon. Animals fed SUDCA developed 50% fewer tumors than controls fed a regular diet, and 55% of the animals fed SUDCA were completely tumor-free. This natural bile acid was shown to have greater efficacy than a related bile acid UDCA that has been in recent clinical trials as a candidate for the prevention of colon cancer in patients with colonic polyps. These findings now mean that a concerted program to perform Phase II and Phase III clinical trials of SUDCA will be started by Axcan Pharma in the next year after completion of the required FDA approved safety/toxicity studies. Colon cancer is the second most common cancer in the Western world and methods of primary and secondary prevention will have major global implications. Our research studies in animal models have demonstrated beneficial effects on bile flow that support a potential clinical utility in liver diseases. Intravenous safety/toxicity studies are being carried out as a prelude to future clinical trials of SUDCA in liver diseases thereby expanding the patented technology.

GRANTS, CONTRACTS AND INDUSTRY AGREEMENTS

Grant and Contract Awards

	Current Year Direct	\$0
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Industry Contracts		
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Setchell, K		
Axcan Pharma		\$192,500
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	Current Year Direct Receipts	\$192,500
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	TOTAL	\$192,500
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PUBLICATIONS

1. Bucuvalas JC, **Setchell KD**. Bile acid metabolism during development. In: Polin RA, Fox WW, Abman SH, editors. Fetal and neonatal physiology 3rd ed. Philadelphia, Pa.: W.B. Saunders Co.; 2004. p. 1179-1185.
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3. Erdman JW, Jr., Badger TM, Lampe JW, **Setchell KDR**, Messina M. Not all soy products are created equal: caution needed in interpretation of research results. *J Nutr* 2004;134(5):1229S-1233S.
4. Kritchevsky D, Tepper SA, Czarenecki SK, Wolfe B, **Setchell KD**. Serum and aortic levels of phytoosterols in rabbits fed sitosterol or sitostanol ester preparations. *Lipids* 2003;38(11):1115-8.
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6. Lephart ED, **Setchell KD**, Handa RJ, Lund TD. Behavioral Effects of Endocrine-disrupting Substances: Phytoestrogens. *ILAR J* 2004;45(4):443-54.
7. Lund TD, Munson DJ, Haldy ME, **Setchell KD**, Lephart ED, Handa RJ. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod* 2004;70(4):1188-95.
8. Messina M, Erdman J, Jr., **Setchell KD**. Introduction to and perspectives from the Fifth International Symposium on the Role of Soy in Preventing and Treating Chronic Disease. *J Nutr* 2004;134(5):1205S-1206S.
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10. **Setchell KD**, Cole SJ. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. *J Agric Food Chem* 2003;51(14):4146-55.
11. **Setchell KD**, Lydeking-Olsen E. Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. *Am J Clin Nutr* 2003;78(3 Suppl):593S-609S.
12. **Setchell KDR**, O'Connell NC. Disorders of bile acid synthesis and metabolism. In: Walker WA, editor. *Pediatric Gastrointestinal Disease* 4th ed. Hamilton, Ontario: BC Decker; 2004. p. 1308-1343.
13. Smith JL, Lewindon PJ, Hoskins AC, Pereira TN, **Setchell KD**, O'Connell NC, Shepherd RW, Ramm GA. Endogenous ursodeoxycholic acid and cholic acid in liver disease due to cystic fibrosis. *Hepatology* 2004;39(6):1673-82.

14. Thigpen JE, Haseman JK, Saunders HE, **Setchell KD**, Grant MG, Forsythe DB. Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. *Comp Med* 2003;53(6):607-15.
15. Thigpen JE, **Setchell KD**, Saunders HE, Haseman JK, Grant MG, Forsythe DB. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. *ILAR J* 2004;45(4):401-16.