

# REPORT DOCUMENTATION PAGE

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# IN VITRO

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VOLUME 30A NUMBER 3 PART II MARCH 1994

DEVELOPMENTAL

BIOLOGY

PROGRAM ISSUE

CONGRESS ON CELL AND TISSUE  
CULTURE

JUNE 4-7, 1994

SHERATON IMPERIAL HOTEL  
RESEARCH TRIANGLE PARK, NC



Journal  
of the  
Tissue  
Culture  
Association

# CONGRESS ON CELL AND TISSUE CULTURE MEETING SUMMARY

JUNE 4-7, 1994 Sheraton Imperial Hotel, Research Triangle Park, NC

	SATURDAY	SUNDAY	JUNE 5	MONDAY	JUNE 6	TUESDAY	JUNE 7	
MORNING	<p>7:30 am-8:30 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>8:00 am-1:00 pm ..... Park Board Room</p> <p>TCA EXECUTIVE BOARD MEETING</p> <p>SPECIAL EDUCATIONAL WORKSHOP Basic Cell and Tissue Culture Methods and Applications ..... Auditorium</p> <p>8:00 am-5:30 pm</p>	<p>7:00 am-6:00 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>8:00-10:00 ..... Empire Ballroom</p> <p>PLENARY SESSION Regulation of Cell and Tissue Differentiation</p> <p>10:00 ..... Exhibit Hall</p> <p>Coffee Break</p> <p>10:00 am-6:00 pm ..... Exhibit Hall</p> <p>Vertebrate Posters Attended As Follows 4:30-6:00</p> <p>10:30</p> <p>Morphogenesis, Plant Cell and Tissue ..... Empire D&amp;E</p> <p>Differentiation ..... Imperial VI-VII</p> <p>Tissue Specific Xenobiotic Metabolism</p> <p>10:30</p> <p>CONTRIBUTED PAPERS</p> <p>Phenotype of Transformed Cells ..... Empire ABC</p> <p>Invertebrate Systems ..... Royal Ballroom</p>	<p>7:00 am-6:00 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>7:00 ..... Bull Durham</p> <p>8:00 ..... Empire ABC</p> <p>SPECIAL SYMPOSIUM</p> <p>CELL CULTURE MODELS OF SENSESCENCE</p> <p>8:00</p> <p>SESSIONS-IN-DEPTH</p> <p>The Role of Molecular Biology in Cellular Toxicology ..... Imperial VI-VII</p> <p>Progress in Marine Invertebrate Cell Culture ..... Royal Ballroom</p> <p>10:00</p> <p>CONTRIBUTED PAPERS</p> <p>Plant Regeneration ..... Empire CD</p> <p>COFFEE BREAK</p> <p>10:00</p> <p>EXHIBITS AND POSTERS</p> <p>Cellular Toxicology Posters Attended As Follows 4:30-6:00</p> <p>10:30</p> <p>SESSIONS-IN-DEPTH</p> <p>The State of the Art for Transformation Systems: What's New/What Works ..... Empire ABC</p> <p>In Vitro Assessment of Transport Mechanism in Mammalian Cells ..... Empire CD</p> <p>10:30</p> <p>CONTRIBUTED PAPERS</p> <p>Growth and Senescence ..... Imperial VI-VII</p>	<p>7:00 am-6:00 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>8:00 ..... Empire Ballroom</p> <p>SPECIAL JOINT SYMPOSIUM Cellular Toxicology and Vertebrate Blood-Brain Barrier Research ..... Empire Ballroom</p> <p>8:00</p> <p>CONTRIBUTED PAPERS</p> <p>Transformation ..... Imperial VI &amp; VII</p> <p>COFFEE BREAK</p> <p>10:00</p> <p>EXHIBITS AND POSTERS</p> <p>Plant Posters Attended As Follows 10:30-12:30</p> <p>10:30</p> <p>CONTRIBUTED PAPERS</p> <p>Differentiation and its Modulation ..... Empire CD</p> <p>10:30</p> <p>SESSIONS-IN-DEPTH</p> <p>The Role of Cell Mediators in the Toxic Response ..... Imperial VI-VII</p>	<p>7:00 am-6:00 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>7:00 ..... Bull Durham</p> <p>8:00 ..... Empire ABC</p> <p>SPECIAL SYMPOSIUM</p> <p>CELL CULTURE MODELS OF SENSESCENCE</p> <p>8:00</p> <p>SESSIONS-IN-DEPTH</p> <p>The Role of Molecular Biology in Cellular Toxicology ..... Imperial VI-VII</p> <p>Progress in Marine Invertebrate Cell Culture ..... Royal Ballroom</p> <p>10:00</p> <p>CONTRIBUTED PAPERS</p> <p>Plant Regeneration ..... Empire CD</p> <p>COFFEE BREAK</p> <p>10:00</p> <p>EXHIBITS AND POSTERS</p> <p>Cellular Toxicology Posters Attended As Follows 4:30-6:00</p> <p>10:30</p> <p>SESSIONS-IN-DEPTH</p> <p>The State of the Art for Transformation Systems: What's New/What Works ..... Empire ABC</p> <p>In Vitro Assessment of Transport Mechanism in Mammalian Cells ..... Empire CD</p> <p>10:30</p> <p>CONTRIBUTED PAPERS</p> <p>Growth and Senescence ..... Imperial VI-VII</p>	<p>7:00 am-6:00 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>7:00 ..... Bull Durham</p> <p>8:00 ..... Empire ABC</p> <p>SPECIAL JOINT SYMPOSIUM Cellular Toxicology and Vertebrate Blood-Brain Barrier Research ..... Empire Ballroom</p> <p>8:00</p> <p>CONTRIBUTED PAPERS</p> <p>Transformation ..... Imperial VI &amp; VII</p> <p>COFFEE BREAK</p> <p>10:00</p> <p>EXHIBITS AND POSTERS</p> <p>Plant Posters Attended As Follows 10:30-12:30</p> <p>10:30</p> <p>CONTRIBUTED PAPERS</p> <p>Differentiation and its Modulation ..... Empire CD</p> <p>10:30</p> <p>SESSIONS-IN-DEPTH</p> <p>The Role of Cell Mediators in the Toxic Response ..... Imperial VI-VII</p>	<p>7:00 am-6:00 pm ..... Imperial Foyer</p> <p>REGISTRATION</p> <p>7:00 ..... Bull Durham</p> <p>8:00 ..... Empire ABC</p> <p>SPECIAL SYMPOSIUM</p> <p>CELL CULTURE MODELS OF SENSESCENCE</p> <p>8:00</p> <p>SESSIONS-IN-DEPTH</p> <p>The Role of Molecular Biology in Cellular Toxicology ..... Imperial VI-VII</p> <p>Progress in Marine Invertebrate Cell Culture ..... Royal Ballroom</p> <p>10:00</p> <p>CONTRIBUTED PAPERS</p> <p>Plant Regeneration ..... Empire CD</p> <p>COFFEE BREAK</p> <p>10:00</p> <p>EXHIBITS AND POSTERS</p> <p>Cellular Toxicology Posters Attended As Follows 4:30-6:00</p> <p>10:30</p> <p>SESSIONS-IN-DEPTH</p> <p>The State of the Art for Transformation Systems: What's New/What Works ..... Empire ABC</p> <p>In Vitro Assessment of Transport Mechanism in Mammalian Cells ..... Empire CD</p> <p>10:30</p> <p>CONTRIBUTED PAPERS</p> <p>Growth and Senescence ..... Imperial VI-VII</p>	
	AFTERNOON	<p>2:00-3:30 ..... Empire D</p> <p>3:00 ..... Exhibit Hall</p> <p>4:00-6:00 ..... Empire B</p> <p>TCA Council Meeting</p> <p>Set Up Posters</p> <p>TCA History Society Program</p>	<p>DEADLINE-MONDAY, 12:00 NOON</p> <p>Banquet Vouchers MUST Be Exchanged for Banquet Ticket If You Plan To Attend</p> <p>1:00-2:30 ..... Crown A</p> <p>1995 Program Committee Meeting</p> <p>2:30</p> <p>SESSIONS-IN-DEPTH</p> <p>Retinoids as Regulators of Gene Expression ..... Empire ABC</p> <p>Recent Progress in Transformation Systems ..... Royal Ballroom</p> <p>New Approaches to Environmental Testing Using Inducible Cellular Defenses ..... Imperial VI-VII</p> <p>Morphogenesis: Hormonal Manipulation of Differentiation ..... Empire D&amp;E</p> <p>3:30</p> <p>Membership Committee ..... Park Board Room</p> <p>4:30-5:30 ..... Crown A</p> <p>Cell Culture Standardization</p>	<p>DEADLINE-MONDAY, 12:00 NOON</p> <p>Banquet Vouchers MUST Be Exchanged for Banquet Ticket If You Plan To Attend</p> <p>1:30</p> <p>Strategic Long-Range Planning Meeting ..... Park Board Room</p> <p>2:30</p> <p>SESSIONS-IN-DEPTH</p> <p>Safety of Genetically Engineered Plants: Science and Perceptions ..... Empire D&amp;E</p> <p>Innovations in Non-Invasive Measurement ..... Empire ABC</p> <p>4:30-5:30 ..... Bull Durham</p> <p>Vertebrate Division Business Meeting</p> <p>5:30</p> <p>Publications Committee Meeting ..... Park Board Room</p>	<p>DEADLINE-MONDAY, 12:00 NOON</p> <p>Banquet Vouchers MUST Be Exchanged for Banquet Ticket If You Plan To Attend</p> <p>1:30</p> <p>Strategic Long-Range Planning Meeting ..... Park Board Room</p> <p>2:30</p> <p>SESSIONS-IN-DEPTH</p> <p>Safety of Genetically Engineered Plants: Science and Perceptions ..... Empire D&amp;E</p> <p>Innovations in Non-Invasive Measurement ..... Empire ABC</p> <p>4:30-5:30 ..... Bull Durham</p> <p>Vertebrate Division Business Meeting</p> <p>5:30</p> <p>Publications Committee Meeting ..... Park Board Room</p>	<p>DEADLINE-MONDAY, 12:00 NOON</p> <p>Banquet Vouchers MUST Be Exchanged for Banquet Ticket If You Plan To Attend</p> <p>1:30</p> <p>Strategic Long-Range Planning Meeting ..... Park Board Room</p> <p>2:30</p> <p>SESSIONS-IN-DEPTH</p> <p>Safety of Genetically Engineered Plants: Science and Perceptions ..... Empire D&amp;E</p> <p>Innovations in Non-Invasive Measurement ..... Empire ABC</p> <p>4:30-5:30 ..... Bull Durham</p> <p>Vertebrate Division Business Meeting</p> <p>5:30</p> <p>Publications Committee Meeting ..... Park Board Room</p>	<p>DEADLINE-MONDAY, 12:00 NOON</p> <p>Banquet Vouchers MUST Be Exchanged for Banquet Ticket If You Plan To Attend</p> <p>1:30</p> <p>Strategic Long-Range Planning Meeting ..... Park Board Room</p> <p>2:30</p> <p>SESSIONS-IN-DEPTH</p> <p>Safety of Genetically Engineered Plants: Science and Perceptions ..... Empire D&amp;E</p> <p>Innovations in Non-Invasive Measurement ..... Empire ABC</p> <p>4:30-5:30 ..... Bull Durham</p> <p>Vertebrate Division Business Meeting</p> <p>5:30</p> <p>Publications Committee Meeting ..... Park Board Room</p>	<p>DEADLINE-MONDAY, 12:00 NOON</p> <p>Banquet Vouchers MUST Be Exchanged for Banquet Ticket If You Plan To Attend</p> <p>1:30</p> <p>Strategic Long-Range Planning Meeting ..... Park Board Room</p> <p>2:30</p> <p>SESSIONS-IN-DEPTH</p> <p>Safety of Genetically Engineered Plants: Science and Perceptions ..... Empire D&amp;E</p> <p>Innovations in Non-Invasive Measurement ..... Empire ABC</p> <p>4:30-5:30 ..... Bull Durham</p> <p>Vertebrate Division Business Meeting</p> <p>5:30</p> <p>Publications Committee Meeting ..... Park Board Room</p>
		<p>8:00-10:00 ..... Exhibit Hall</p> <p>OPENING RECEPTION</p>	<p>6:00-7:30 pm ..... Crown Ballroom</p> <p>STUDENT SOCIAL</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Primary Culture of Differentiated Cells ..... Royal Ballroom</p> <p>Cell and Tissue Culturing in NASA Bioreactors ..... Empire ABC</p> <p>Advances in Automation for Micropropagation ..... Empire D&amp;E</p> <p>Com Transformation ..... Auditorium</p>	<p>6:00-7:30 pm ..... Crown Ballroom</p> <p>STUDENT SOCIAL</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Primary Culture of Differentiated Cells ..... Royal Ballroom</p> <p>Cell and Tissue Culturing in NASA Bioreactors ..... Empire ABC</p> <p>Advances in Automation for Micropropagation ..... Empire D&amp;E</p> <p>Com Transformation ..... Auditorium</p>	<p>6:00-7:30 pm ..... Crown Ballroom</p> <p>STUDENT SOCIAL</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Primary Culture of Differentiated Cells ..... Royal Ballroom</p> <p>Cell and Tissue Culturing in NASA Bioreactors ..... Empire ABC</p> <p>Advances in Automation for Micropropagation ..... Empire D&amp;E</p> <p>Com Transformation ..... Auditorium</p>	<p>6:00-7:30 pm ..... Crown Ballroom</p> <p>STUDENT SOCIAL</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Primary Culture of Differentiated Cells ..... Royal Ballroom</p> <p>Cell and Tissue Culturing in NASA Bioreactors ..... Empire ABC</p> <p>Advances in Automation for Micropropagation ..... Empire D&amp;E</p> <p>Com Transformation ..... Auditorium</p>	<p>6:00-7:30 pm ..... Crown Ballroom</p> <p>STUDENT SOCIAL</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Primary Culture of Differentiated Cells ..... Royal Ballroom</p> <p>Cell and Tissue Culturing in NASA Bioreactors ..... Empire ABC</p> <p>Advances in Automation for Micropropagation ..... Empire D&amp;E</p> <p>Com Transformation ..... Auditorium</p>	<p>6:00-7:30 pm ..... Crown Ballroom</p> <p>STUDENT SOCIAL</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Primary Culture of Differentiated Cells ..... Royal Ballroom</p> <p>Cell and Tissue Culturing in NASA Bioreactors ..... Empire ABC</p> <p>Advances in Automation for Micropropagation ..... Empire D&amp;E</p> <p>Com Transformation ..... Auditorium</p>
EVENING			<p>5:00 ..... Bull Durham</p> <p>Cellular Toxicology Committee</p>	<p>7:00</p> <p>TCA Plant Cellular and Developmental Biology Division Business Meeting ..... Holiday Inn, Raleigh/Durham</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Law and Liability in the Laboratory ..... Auditorium</p> <p>A Feeling for the Organism: Novel Approaches to the Culture of Difficult Invertebrate Cells ..... Royal Ballroom</p> <p>BioCoat® Cell/Environments: Construction of In Vitro Models for Hepatocytes and Enterocytes ..... Empire C&amp;D</p> <p>8:00</p> <p>TCA Plant Cellular and Developmental Biology Division Social ..... Holiday Inn, Raleigh-Durham</p> <p>9:00</p> <p>Invertebrate Division Business Meeting ..... Royal Ballroom</p>	<p>7:00</p> <p>TCA Plant Cellular and Developmental Biology Division Business Meeting ..... Holiday Inn, Raleigh/Durham</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Law and Liability in the Laboratory ..... Auditorium</p> <p>A Feeling for the Organism: Novel Approaches to the Culture of Difficult Invertebrate Cells ..... Royal Ballroom</p> <p>BioCoat® Cell/Environments: Construction of In Vitro Models for Hepatocytes and Enterocytes ..... Empire C&amp;D</p> <p>8:00</p> <p>TCA Plant Cellular and Developmental Biology Division Social ..... Holiday Inn, Raleigh-Durham</p> <p>9:00</p> <p>Invertebrate Division Business Meeting ..... Royal Ballroom</p>	<p>7:00</p> <p>TCA Plant Cellular and Developmental Biology Division Business Meeting ..... Holiday Inn, Raleigh/Durham</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Law and Liability in the Laboratory ..... Auditorium</p> <p>A Feeling for the Organism: Novel Approaches to the Culture of Difficult Invertebrate Cells ..... Royal Ballroom</p> <p>BioCoat® Cell/Environments: Construction of In Vitro Models for Hepatocytes and Enterocytes ..... Empire C&amp;D</p> <p>8:00</p> <p>TCA Plant Cellular and Developmental Biology Division Social ..... Holiday Inn, Raleigh-Durham</p> <p>9:00</p> <p>Invertebrate Division Business Meeting ..... Royal Ballroom</p>	<p>7:00</p> <p>TCA Plant Cellular and Developmental Biology Division Business Meeting ..... Holiday Inn, Raleigh/Durham</p> <p>7:00</p> <p>WORKSHOPS</p> <p>Law and Liability in the Laboratory ..... Auditorium</p> <p>A Feeling for the Organism: Novel Approaches to the Culture of Difficult Invertebrate Cells ..... Royal Ballroom</p> <p>BioCoat® Cell/Environments: Construction of In Vitro Models for Hepatocytes and Enterocytes ..... Empire C&amp;D</p> <p>8:00</p> <p>TCA Plant Cellular and Developmental Biology Division Social ..... Holiday Inn, Raleigh-Durham</p> <p>9:00</p> <p>Invertebrate Division Business Meeting ..... Royal Ballroom</p>	

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JOURNAL OF  
**Tissue  
Culture  
Methods**

TISSUE CULTURE ASSOCIATION MANUAL OF CELL, TISSUE, AND ORGAN CULTURE PROCEDURES

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# 1994 CONGRESS ON CELL AND TISSUE CULTURE

## Schedule of Functions

Date	Type of Function	Room
<b>Saturday, June 4</b>		
8:00 am	TCA Executive Board Meeting	Park Board Room
8:00 am	Special Educational Workshop	Auditorium
2:00 pm	TCA Council Meeting	Empire D
4:00 pm	TCA History Society Meeting	Empire B
8:00 pm	Congress Opening Reception	Exhibit Hall
<b>Sunday, June 5</b>		
10:00 am	<b>Exhibits and Posters</b> (10:00 am to 6:00 pm)	Exhibit Hall
1:00 pm	1995 Program Committee Meeting	Crown Ballroom
3:30 pm	Membership Committee	Park Board Room
4:30 pm	Cell Culture Standardization	Crown Ballroom
5:00 pm	Cellular Toxicology Committee	Bull Durham
6:00 pm	Student Social	Crown Ballroom
<b>Monday, June 6</b>		
7:00 am	TCA Plant Program Breakfast Meeting	Bull Durham
10:00 am	<b>Exhibits and Posters</b> (10:00 am to 6:00 pm)	Exhibit Hall
12:30 pm	Laboratory Materials and Biosafety Committee	Crown Ballroom
1:30 pm	Strategic Long-Range Planning Meeting	Park Board Room
4:30 pm	Vertebrate Division Business Meeting	Bull Durham
5:30 pm	Publications Committee Meeting	Park Board Room
7:00 pm	TCA Plant Cellular and Developmental Division Business Meeting	Holiday Inn-Raleigh Durham
8:00 pm	TCA Plant Cellular and Developmental Division Social	Holiday Inn-Raleigh Durham
9:00 pm	Invertebrate Division Business Meeting	Royal Ballroom
<b>Tuesday, June 7</b>		
10:00 am	<b>Posters</b> (10:00 to 3:00)	Exhibit Hall
10:00 am	<b>Exhibits</b> (10:00 to 3:00)	Exhibit Hall
1:30 pm	Development Committee	Park Board Room
4:30 pm	TCA Business Meeting	Royal Ballroom
8:00 pm	Congress Banquet	Poolside

**NOTE:** Additions and changes to functions will be posted on a bulletin board located in the registration area.  
Please check the bulletin board daily.

### PLAN TO VISIT THE EXHIBITS

Sunday	10:00 am - 6:00 pm
Monday	10:00 am - 6:00 pm
Tuesday	10:00 am - 5:00 pm

Morning coffee breaks Sunday, Monday, and Tuesday  
Cash bar will begin at 4:30 on Sunday and Monday





PROGRAM

SUNDAY, JUNE 5

10:30 am - 12:30 pm

SESSION-IN-DEPTH  
(See Abstracts on Page 38)

Imperial VI-VII

Sponsored by DuPont Merck Pharmaceutical Company

- 10:30 T **Tissue Specific Xenobiotic Metabolism**  
Convener: Shuet-Hing Lee Chiu, Merck Laboratories
- 10:45 T-1 Drug Metabolism in Primary Cultures of Adult Human Hepatocytes  
P. MAUREL
- 11:20 T-2 Metabolic Activation of NNK by Human Cytochromes P450 Stably Expressed in Mammalian Cells  
R. LANGENBACH, M. Cunningham, H. Tiano, and C. Crespi
- 11:55 T-3 Sex- and Tissue-Specific Expression of Highly Homologous Mouse Cytochrome P450s 2a-4 and 2a-5  
M. NEGISHI

10:30 am - 11:30 am

CONTRIBUTED PAPER SESSION  
(See Abstracts on Pages 56-57)

Royal Ballroom

- I **Invertebrate Systems**  
Convener: Timothy J. Kurtti, University of Minnesota
- 10:30 I-1001 Juvenile Hormone Production and Cell Proliferation by Cockroach Corpora Allata *In Vitro*  
G.L. HOLBROOK, W.-H. Tsai, A.-S. Chiang, and C. Schal
- 10:45 I-1003 Replication of *Choristoneura biennis* Entomopoxvirus in *Choristoneura fumiferana* (Lepidoptera: Tortricadae) Cell Lines  
S.S. SOHI, B.M. Arif, B.J. Cook, J.A. MacDonald, and G.F. Caputo
- 11:00 I-1004 *In Vitro* Primary Cell Culture of *Hermisenda crassicornis* Neurons  
A.M. KUZIRIAN, P.J.S. Smith, and C. Collin

10:30 am - 12:00 am

CONTRIBUTED PAPER SESSION  
(See Abstracts on Pages 94-95)

Empire ABC

- V **Phenotype of Transformed Cells**  
Convener: Richard Heller, University of So. Florida
- 10:30 V-1001 Detection of Metastatic Melanoma by Reverse-Transcription PCR  
R. HELLER, X. Wang, N. VanVoorhis, C.W. Cruse, D. Reintgen
- 10:45 V-1002 Regulation of Differentiation in A549 Malignant Pneumocytes by Paracrine Growth Factors  
C. McCormick, R.I. FRESHNEY, and L. Evans
- 11:00 V-1003 DNA Fingerprinting of Human Tumor Lines Using Minisatellite Probes  
Y.A. Reid, P. McClintock, C. WHITE, and M. Rossano-Theurer
- 11:15 V-1004 Transformation of Lymphatic Endothelial Cells by Infection with SV40 DNA  
L.V. LEAK
- 11:30 V-1005 Specific Binding of GS-I-B4 Lectin to Culture Human Malignant Mesothelioma Cells-- Isolation and Partial Characterization of Mesothelioma Specific Glyco-proteins  
K.H. BERGHÄUSER, B. Knoblauch, D. Linder, C.M. Heinrichs, A. Schulz

**\*\*NOTICE\*\***

Deadline: Monday 12:00 noon  
Exchange Banquet Voucher For Tuesday Banquet Ticket.  
Voucher Must Be Exchanged For A Banquet Ticket If You Plant To Attend  
The Banquet Seating Is Limited To Banquet Ticket Holders ONLY!

## SUNDAY, JUNE 5

2:30 pm - 4:30 pm

SESSION-IN-DEPTH  
(See Abstracts on Page 45)

Empire ABC

## V Retinoids as Regulators of Gene Expression In Vitro

Convener: Peter J. Davies, The University of Texas

Retinoids are natural or synthetic compounds structurally related to Vitamin A. They are extremely potent regulators of gene expression in mammalian cells. Molecular studies have identified families of retinoid receptors (RARs and RXRs) that combine with retinoids to regulate the transcription of a variety of genes. The focus of this symposium will be to examine the interaction of the retinoids and their receptors and their effects on the expression of genes controlling cell proliferation, differentiation, and death.

2:30		Introduction (P.J. Davies)
2:35		Retinoid-Regulated Expression of Tissue Transglutaminase in Apoptotic Cells
	V-1	P.J. DAVIES, L. Nagy, V. Thomazy, and R. Chandraratna
3:05		Regulation of Gene Expression by Retinoids During Squamous Cell Differentiation
	V-2	A.M. JETTEN
3:35		Strategies Towards Studying Retinoid Signaling in Cells and Mouse Embryos
	V-3	E. LINNEY, M. Colbert, A. Darrow, T.M. Underhill, D. Cash, Q. Liu, L. Kotch, B. Smith, G.A. Johnson, and A. LaMantia
3:55		RARs and RXRs Are Required for Prevention of Activation-Induced T Cell Apoptosis by Retinoic Acid
	V-4	J.D. ASHWELL, Y. Yang, and R. Heyman

2:30 pm - 4:35 pm

SESSION-IN-DEPTH  
(See Abstracts on Pages 33-34)

Empire D&amp;E

## P Morphogenesis: Hormonal Manipulation of Differentiation

Convener: T.S. Rangan, Phytogen

Plant cell and tissue differentiation in vivo and in vitro is mediated by hormones. Cytokinins have been implicated in cell division and shoot morphogenesis whereas auxins have been implicated in cell division, cell expansion, and root morphogenesis. Additionally, gibberellins have been shown to be involved in cell expansion and flowering. Hormonal control of in vitro differentiation of phloem and tracheary elements has been intensely studied by a number of laboratories. Dr. R.D. Sjölund has shown that in vitro development of sieve elements and companion cells, elements of phloem, parallels that seen in whole plants. Dr. G.F. Peter and I.M. Sussex, working with *Zinnia elegans* leaf mesophyll that differentiate into tracheary cells in vitro, will discuss their efforts to determine whether genes expressed in these differentiating cells are similar to those differentiating from cells of meristematic origin. Similarly, in vitro embryo and meristem development is controlled by hormonal manipulation as shown by Qili Feng et al. K. Padmanabhan et al. have shown that lack of organized shoot development during somatic embryogenesis can be attributed to lack of an organized meristem, flattened apical meristem, sparsity of dividing cells, and diffuse meristematic activity. Similar studies of these abnormalities, leading to population heterogeneity of somatic embryos, have allowed the implementation of a pattern recognition system through image analysis that will aid in developing strategies for large-scale micropropagation.

2:30		Introduction (T.S. Rangan)
2:35		Phloem Differentiation in Plant Tissue Cultures.
	P-5	R.D. SJÖLUND
3:05		Developmental Pathway(s) of Xylem Tracheary Element Differentiation
	P-6	G.F. PETER and I.M. Sussex
3:35		Developmental Kinetics of Carrot Somatic Embryo Culture
	P-7	C.-M. CHI, H. Vits, E.J. Staba, T.J. Cooke, and W.-S. Hu
3:55		A Comparison of External Morphology and Internal Anatomy of Shoot Forming and Non-Shoot Forming Somatic Embryos of Sweetpotato ( <i>Ipomea batatas</i> (L.) Lam)
	P-8	K. PADMANABHAN, D.J. Cantliffe, R.C. Harrell, and D.B. McConnell
4:15		Peanut Plant Regeneration Through In Vitro Culture of Peg Tips and Ovules of <i>Arachis hypogaea</i> and <i>A. duranensis</i>
	P-9	Q.L. FENG, H.T. Stalker, and H.E. Pattee

# PROGRAM

**SUNDAY, JUNE 5**

**2:30 pm - 5:00 pm**

**SESSION-IN-DEPTH**  
(See Abstracts on Pages 29-30)

**Royal Ballroom**

**I Recent Progress in Transformation Systems**  
*Convener:* Ann Fallon, University of Minnesota

- 2:30 Introduction (A. Fallon)  
2:35 Transformation of Arthropods by Maternal Microinjection  
I-1 M.A. HOY and J.K. Presnail  
3:05 The Use of *mariner* Transposable Elements as Insect Transformation Vectors  
I-2 D.J. LAMPE  
3:35 Expression of Foreign Genes in Tsetse Flies  
I-3 S. AKSOY, C.B. Beard  
4:05 Viral Expression Vectors for Mosquito Cells and Mosquitoes  
I-4 J. CARLSON, K. Olson, S. Higgs, B. Afanasiev, and B. Beaty  
4:35 Transgenic Mosquitoes: Contributions From Cell Culture  
I-5 A.M. FALLON

**2:30 pm - 5:00 pm**

**SESSION-IN-DEPTH**  
(See Abstracts on Pages 38-40)

**Imperial VI-VII**

*Co-Sponsored by Xenometrix, Inc. and StressGen Biotechnologies Corporation*  
*Hosted by Society of Toxicology and the Cellular Toxicology Committee*

**T New Approaches to Environmental Testing Using Inducible Cellular Defenses**  
*Co-Conveners:* Lawrence Hightower, University of Connecticut, and Eugene Elmore, National Institute for the Advancement of In Vitro Science

- 2:30 Overview of the Cellular Functions of Stress Proteins and Their Potential for Use as Molecular Biomarkers  
T-4 L.E. HIGHTOWER  
2:55 Biomonitoring Using An Organism's Own Cellular Stress Response System  
T-5 J.A. RYAN and L.E. Hightower  
3:20 Testing of Xenobiotics and Environmental Samples Using Stress-Inducible Transgenic Strains of the Nematode, *Caenorhabditis elegans*  
T-6 E.P.M. CANDIDO and E.G. Stringham  
3:45 Use of Transcriptional Activation of Stress Response Genes to Establish "Molecular Fingerprints" of Toxicants and Development of a Reference Database  
T-7 C.S. ORSER and S.B. Farr  
4:10 Cytochrome P450 Induction and Inhibition by Planar Halogenated Aromatic Hydrocarbons in a Fish Cell Line: Promise and Pitfalls for Environmental Testing  
T-8 M.E. HAHN  
4:35 CYPIA1 Inductive Responses in Hepatoma Cell Lines as a Tool in Environmental Toxicology  
T-9 D.E. TILLITT

**4:30 pm - 6:00 pm**

**POSTER PRESENTATION**

Vertebrate and Invertebrate Poster Authors Will Be Present  
Sunday, June 5

V-1018 to V-1065 (See List of Posters on Pages 19-21)

**6:00 pm - 7:30 pm**

**STUDENT SOCIAL**  
*Students Are Encouraged To Attend*

**Crown Ballroom**

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PROGRAM

SUNDAY, JUNE 5

7:00 pm - 9:30 pm

**WORKSHOPS**  
(See Abstracts on Page 52)

Imperial VI-VII

**P Automation in Micropropagation**

*Co-Conveners:* Neville Arnold, Agriculture Canada, and Barbara Reed, USDA/ARS

Over the past several years, we have seen major developments in image-guided robots, robotic work stations and systems of environmental control in micropropagation systems for shoot cultures and somatic embryos. These advances are both timely and important because of the competitiveness in the tissue culture industry and the economics of the times. This workshop will therefore present the latest developments in Systems for Automated Environmental Control (Dr. Chieri Kubota), Quality Quantification of Somatic Coffee Embryos Using Machine Vision (Dr. Peter Ling), Global Perspectives on Automation Systems (Dr. Mary Ann Lila Smith) and Micropropagation Automation Utilizing the Liquid/Membrane Approach (Dr. Roy E. Young).

7:00		Introduction N. Arnold and B. Reed
7:05		A Global Perspective on Automation Systems M.A.L. SMITH
7:35	W-4	Somatic Coffee Embryo Quality Quantification Using Machine Vision P.P. LING, Z. Cheng, and D.J. Musacchio
8:05	W-3	Methods and Systems for Automatic Environmental Control T. Kozai and C. KUBOTA
8:35	W-2	Micropropagation Automation Utilizing the Liquid/Membrane Approach R.E. YOUNG

7:00 pm - 10:00 pm

**WORKSHOPS**  
(See Abstracts on Pages 52-55)

Empire ABC

**V Cell and Tissue Culturing in NASA Bioreactors**

*Co-Conveners:* Neal Pellis, University of Texas, and Glenn Spaulding, Johnson Space Center

7:00		Perfusion in a NASA Cell Culture System G.F. SPAULDING
7:15	W-6	Cell and Tissue Culture Research Within NASA Life Science K. SCRIBNER
7:30	W-7	Effects of Shear on <i>In Vitro</i> Chondrogenesis L.E. FREED, G. Vunjak, J.K. Blum, J. Emmanuel
7:45	W-8	Cultured Human Epithelial and Smooth Muscle Cells E.M. LEVINE, T.J. Goodwin, T.L. Prewett, and G.F. Spaulding
8:00	W-9	Culture of Cardiac and Skeletal Muscle Cells in the NASA Bioreactor Vessel N.A. Schroedl, R.E. Akins, G.R. Molnar, S.R. Gonda, and C.R. HARTZELL
8:15	W-10	CEA Production in Human Colon Cancer J. MILBURN JESSUP, W. Fitzgerald, J. Polanec, R. Ford, T. Goodwin, G.F. Spaulding, and D. Brown
8:30	W-11	Growth and Gene Expression in Human Ovarian Cancer J.L. BECKER, T.L. Prewett, T.J. Goodwin, and G.F. Spaulding
8:45	W-12	Microgravity Affects Human Prostrate Cancer Growth and Differentiation: A Preliminary Report L.W.K. CHUNG, T.J. Goodwin, H.Y.E. Zhou, T.L. Prewett, G.F. Spaulding, and S.M. Chang
9:00	W-13	Metabolic Activity of Insect Cells Cultured in Simulated Microgravity K. Francis, N. Johnson, K. O'CONNOR and G. Spaulding
9:15	W-14	Human Lymphocyte Locomotion in Randomized Gravity N.R. PELLIS, T.J. Goodwin, D. Risin, B.W. McIntyre, R.P. Pizzini, D. Cooper, T.L. Prewett, and G.F. Spaulding

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PROGRAM

SUNDAY, JUNE 5

7:00 pm

WORKSHOP

Royal Ballroom

**V Primary Culture Differentiated Cells**

*Convener:* Colette Rudd, SRI

Primary cell culture is an important source of differentiated cells for *in vitro* research. Cells from many tissues have been isolated and characterized in primary cultures. Techniques to characterize the differentiated cells include immunostaining, enzyme assays, electrophysiology, morphology, and *in situ* hybridization. Hormones and other components of the culture media are usually essential for optimum expression of the unique functions of the cells. In the past few years, there has been increasing recognition that the spatial environment of the cells is also an important factor in maintaining cellular differentiation. Applications for differentiated cells in culture, especially human cells, include pharmacological studies of efficacy, metabolism and toxicity of chemicals, synthesis of novel proteins, cell transplantation, and gene therapy. This workshop will include an initial overview of primary cell culture followed by presentations focusing on characterization of cells from specific tissues and applications of these culture systems.

Techniques for Culture and Characterization of Differentiated Cells

C.J. RUDD

Development of Artificial Liver With Primary Hepatocytes

A.P. LI

Culture and Characterization of Normal Colonic Epithelial Cells: Response to Inflammatory Cytokines

A. VIDRICH

7:00 pm

WORKSHOP

Auditorium

**P Corn Transformation**

*Convener:* Paul Zankowski, Harris Moran Seed Company

Transformation systems for expressing genes in cells of corn and regenerating transgenic plants have been in place since 1986. Several different transformation approaches have been developed since that time, each with inherent benefits, difficulties, and nuances. The success or failure of each transformation system is dependent upon many biotic and abiotic factors. The goal of this workshop is to discuss those factors that provide the foundation for a successful corn transformation system. Representatives from some of the major industry players in this field will discuss the diverse approaches toward corn transformation.

- 7:00 High Frequency Transformation of Maize By Microprojectile Bombardment of Immature Embryos  
J. DAWSON
- 7:30 Transgenic Maize via a Novel Delivery With an Appropriate Target Tissue  
M. MILLER
- 8:00 Corn Popping: Biolistic Transformation of Corn Callus  
T. JONES
- 8:30 Biolistic vs. Electroporation Gene Delivery Into Immature Maize Embryos  
D. SONGSTAD
- 9:00 Commercial-Scale Production of Transgenic Corn  
C. ARMSTRONG

# PROGRAM

**MONDAY, JUNE 6**

7:00 am - 6:00 pm

**MEETING REGISTRATION**

**Imperial Foyer**

**\*\*NOTICE\*\***

Deadline: Monday 12:00 noon  
Exchange Banquet Voucher For Tuesday Banquet Ticket.  
Voucher Must Be Exchanged For A Banquet Ticket If You Plan To Attend  
The Banquet Seating Is Limited To Banquet Ticket Holders ONLY!

8:00 am - 10:00 am

**SESSION-IN-DEPTH**

**Royal Ballroom**

(See Abstracts on Pages 30-31)

**I Progress in Marine Invertebrate Cell Culture**  
*Convener:* Lehman Ellis, University of New Orleans

- 8:00 Introduction (L. Ellis)  
8:05 Adhered Tissue From a Hexactinellid Sponge Reveals a Novel Transport System  
I-6 S.P. LEYS  
8:40 Recent Progress in Marine Sponge Cell Culture  
I-7 S.A. POMPONI and R. Willoughby  
9:15 Novel Marine Alkaloids That Regulate Cell Growth and Differentiation  
I-8 N.R. Shochet, D. Olchovsky, and I. SPECTOR  
9:55 I-9 TBA (B. Rinkervich)

8:00 am - 10:00 am

**CONTRIBUTED PAPERS**

**Empire C&D**

(See Abstracts on Pages 58-59)

**P Regeneration/In Vitro Culture**  
*Convener:* Prem Kahlon, Tennessee University

- 8:00 High Efficiency of Regeneration of Peanut Using a Nonimbibed Immature Leaflet Culture Method  
P-1001 S.D. UTOMO, A.K. Weissinger, H.T. Stalker, T.G. Isleib  
8:15 Adventitious Shoot Regeneration from Cotyledons, Leaf Tissue, and Cell Suspension in Hackberry (*Celtis Occidentalis* L.)  
P-1002 Z.-M. CHENG and N.-Q. Shi  
8:30 Tissue Culture of Ginkgo  
P-1003 N.D. CAMPER, R.J. Keese, and D.E. Wedge  
8:45 *In Vitro* Culture of Scots Pine (*Pinus sylvestris* 'Ladoga') from Dormant Bud Explants  
P-1004 T. Peh and K. PRUSKI  
9:00 Microcuttings of *Taxus x media* cv. *Hicksii*  
P-1005 R.M. CERDEIRA, J.D. McChesney, and C. Burandt, Jr.  
9:15 *In Vitro* Germination of *Musa velutina* Seeds  
P-1006 N. PANCHOLI, A. Wetten, and P.D.S. Caligari  
9:30 Influence of a Blue-Green Algal Extract on Shoot Multiplication and Rooting of *Vitis rotundifolia*  
P-1007 A.M. ALLAM

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PROGRAM

MONDAY, JUNE 6

8:00 am - 10:00 am

**SESSION-IN-DEPTH**  
*(See Abstracts on Pages 46-47)*

Empire ABC

**V Cell Culture Models of Senescence**  
*Convener: James R. Smith, Baylor College of Medicine*

The Session-in-Depth on Cell Culture Models of Senescence is designed to address our understanding of the phenomenon of cell senescence in fibroblasts, endothelial cells, T-cells, myocytes, and adrenocortical cells in vitro. Each cellular system has provided unique insights into the mechanism(s) that play a role in the process of cellular aging.

- 8:00 Introduction (J. Smith)  
8:05 Functional Activity of Long-Term Normal and SV40 Transfected T Lymphocyte Cultures  
V-5 D.M. MURASKO and Q.C. Ryan  
8:25 Senescence of Adrenocortical Cell in Culture  
V-6 P.J. HORNSBY  
8:45 The Fibroblast Growth Factor (FGF)-1 Signaling Pathway Is Defective In the Interleukin (IL)-  
1 $\alpha$ -Mediated Pathway of Human Umbilical Vein Endothelial Cell (HUVEC) Senescence  
V-7 X. Hu, S. Garfinkel, and T. MACIAG  
9:05 Cellular Senescence and Cell Cycle Regulators  
V-8 M. NAKANISHI, S. Venable, and J.R. Smith  
9:25 Closing Remarks

8:00 am - 10:00 am

**SESSION-IN-DEPTH**  
*(See Abstracts on Pages 40-41)*

Empire ABC

*Sponsored by Industrial In Vitro Toxicology Group*

**T The Role of Molecular Biology in Cellular Toxicology**  
*Convener: Jack Lipman, Hoffman-LaRoche*

- 8:00 Introduction (J. Lipman)  
8:15 Molecular Approaches to Retinoid-Induced Teratogenesis  
T-10 J.F. GRIPPO, H.J. Kim, M.B. Zhang, and D.A. Lucas  
8:40 Novel Molecular Approaches to Toxicology and Drug Discovery  
T-11 S.B. FARR  
9:05 Hepatic Peroxisome Proliferation: Mechanisms, Species Differences and *In Vitro/In Vivo*  
Correlations  
T-12 B.G. LAKE

10:00 am

**COFFEE BREAK**

Exhibit Hall

10:00 am-6:00 pm

**EXHIBITS AND POSTERS**  
*Posters must be removed from Exhibit hall  
by 5:00 pm Tuesday, June 7*

Exhibit Hall

## MONDAY, JUNE 6

10:30 am - 12:30 pm

CONTRIBUTED PAPERS  
(See Abstracts on Pages 95-97)

Imperial VI-VII

V **Growth and Senescence***Convener:* Gertrude C. Buehring, University of California

- 10:30 V-1006 Differential Expression of Genes Regulating Redox State During In Vitro and In Vivo Aging  
B. KEOGH, R.G. Allen, G.S. Gerhard, R. Pignolo, J. Horton, and V.J. Cristofalo
- 10:45 V-1007 Lifespan of Bovine Mammary Epithelial Cells In Vitro  
G.C. BUEHRING, R.L. Neiswander, G.L. Niermann, C.A. Sweeney, M.F. McGrath, F.L. Schanbacher
- 11:00 V-1008 Transfection of Fetal Human Articular Chondrocytes by SV40 Large T Antigen: Increased Life Span and Modification of Collagen Phenotype  
B. BENOIT, S. Thenet-Gauci, P. Penforis, S. Demignot, and M. Adolphe
- 11:15 V-1009 Establishment and Characterization of Immortalized Clones of Acinar Cells From Adult Rat Parotid Glands  
K.N. Prasad, S. Kumar, E. Carvalho, J. Edwards-Prasad, and F.H. La Rosa
- 11:30 V-1010 Cell Growth and Hybridoma Protein Secretion in Gamma-Irradiated Animal Serum Is Equivalent to Matched Nonirradiated Control Serum  
R. FESTEN, B. Alderete, J. Doak, J.D. Keathley, and D.E. Wyatt
- 11:45 V-1011 Magnesium, Glutamine, and Serum as Interactive Agents in the Anchorage Independent Colony Formation of Rat Liver Epithelial Cells  
B.S. HASS, P.L. McDaniel, N.A. Littlefield

10:30 am - 12:30 pm

SESSION-IN-DEPTH  
(See Abstracts on Pages 34-35)

Empire ABC

P **The State of the Art for Transformation Systems: What is New; What Works?***Convener:* Ted Klein, DuPont Agricultural Products

Efficient and reproducible techniques for gene transfer to a number of major crop species have been developed in recent years. This session will highlight some of the latest advances including gene transfer to intact tissues by particle bombardment, electroporation, and silica carbide fibers. The inheritance and stability of expression of transgenes will also be presented, including the latest information on the cosuppression phenomenon.

- 10:30 Introduction (T. Klein)
- 10:35 P-10 Efficient Production of Transgenic Barley Plants and Analysis of Transgene Expression in Progeny  
Y. WAN and P.G. Lemaux
- 11:05 P-11 Production of Fertile Transgenic Maize by Electroporation  
T.M. SPENCER, C.M. Laursen, R.A. Krzyzek, P.C. Anderson, T.R. Adams, and C.E. Flick
- 11:35 P-12 Production of Fertile Transgenic Maize Plants By Silicon Carbide Fiber-Mediated Transformation  
B.R. FRAME, P.R. Drayton, S.V. Bagnall, C.J. Lewnau, W.P. Bullock, H.M. Wilson, J.M. Dunwell, J.A. Thompson, K. Wang
- 12:05 P-13 Genetic Transformation and Gene Suppression in Seed and Vegetatively Propagated Crops  
N. COURTNEY-GUTTERSON

## MONDAY, JUNE 6

10:30 am - 12:30 pm

## SESSION-IN-DEPTH

Empire C&amp;D

*(See Abstracts on Pages 41-42)**Sponsored by Corning Costar Corporation***T In Vitro Assessment of Transport Mechanisms in Mammalian Cells***Convener: Seth Alper, Beth Israel Hospital*

- 10:30 T-13 The AE Anion Exchanger Proteins: Structure, Function, Localization, and Regulation  
S.L. ALPER, M.L. Chernova, Y. Zhang, B.D. Humphreys, A.S. Zolotarev, D. Yannoukakos,  
A. Stuart-Tilley, and L. Jiang
- 11:00 T-14 Cell Culture Models of Cystic Fibrosis Airway Epithelia  
J.R. YANKASKAS
- 11:30 T-15 PTH-Stimulated Calcium Transport by Cultured Kidney Cells Requires Activation of Protein  
Kinase A and Protein Kinase C  
P.A. Friedman, F.A. Gesek, B.A. Coutermarsh, and S.M. Kennedy
- 12:00 T-16 Entry of Cholera Toxin (CT) into the Polarized Human Intestinal Epithelial Cell Line  
W.I. LENCER

2:30 pm - 4:30 pm

## SESSION-IN-DEPTH

Empire ABC

*(See Abstracts on Page 47)***V Innovations in Non-Invasive Measurement of Cells Functions***Convener: Charles Keese, Rensselaer Polytechnic Institute*

The development of modern biosensors is making possible several means to follow the activities of cultured cells in continuous and non-invasive manners. In addition, the electrical output of many of these devices can be readily coupled with laboratory computers. In this manner, more and more studies of cells in culture can be performed with minimal human involvement, as computers both direct the acquisition and carry out the processing of data. In addition to the development of sensors, new culturing devices are also being perfected to reduce human manipulations of cells and to allow their study in remote locations.

In this session, three talks will describe novel non-invasive approaches to monitor metabolism, morphology and motility, and the release of chemical packets from cells in culture. A fourth talk will describe a self-contained system to nourish and study cells under microgravity conditions aboard the Space Shuttle.

- 2:30 Introduction (C. Keese)
- 2:35 V-9 Automated Cell Culture Systems for the Space Shuttle  
W.P. WIESMANN, L.A. Pranger, E.S. Delaplaine, and T.C. Cannon
- 3:05 V-10 Chemical Sensing of Quantal Release of Catecholamines from Cultured Adrenal Cells  
R. Mark Wrightman
- 3:35 V-11 Microphysiometry: Rapid Bioassays Based on Changes in Cell Metabolism  
J. WALLACE PARCE
- 4:05 V-12 ECIS: An Electrical Method to Continuously Monitor Morphology and Motion of Cells in  
Culture  
C.R. KEESE and I. Giaever

MONDAY, JUNE 6

2:30 pm - 4:05 pm

SESSION-IN-DEPTH  
(See Abstracts on Pages 35-36)

Empire D&E

**P Safety of Genetically Engineered Plants: Science and Perceptions**  
*Convener:* Maud Hinchee, Monsanto

In the near future, genetic engineering will be critical for providing new plant varieties which will feed the world. This session will provide valuable insight into how scientists and the public view the safety of genetically engineered crops. Scientific studies on the actual and perceived impacts of genetically engineered crops on the environment as well as on human food and animal feeds will be discussed.

- 2:30 Introduction (M. Hinchee)  
2:35 Approaches to Food and Feed Safety Assessments  
P-14 S.R. PADGETTE, R.L. Fuchs, S.G. Rogers, and D.B. Re  
3:05 Public Perception of Biotechnology  
P-15 T.J. HOBAN  
3:35 The Relative Safety of Transgenic and Non-Transgenic Plants: How Do We Assess It?  
P-16 P.J. DALE

4:15 pm - 5:30 pm

CONTRIBUTED PAPER SESSION  
(See Abstracts on Pages 59-60)

Empire D&E

**P Resistant Plants**  
*Convener:* Martha Wright, Ciba-Geigy Corporation

- 4:15 Screening of *Zea mays* Plants for Phosphinothricin Resistance Using the Chlorophenol Red Test  
P-1008 M.S. WRIGHT, R.D. Shillito, K. Launis, C. Bowman, M. Hill, J. DiMaio  
4:30 Production of Rice Resistant to AHAS-Inhibiting Herbicides  
P-1009 T.P. CROUGHAN  
4:45 Use of Culture Filtrates of *Discula destructiva* to Develop Resistance in *Cornus florida*  
P-1010 D.E. WEDGE and F.H. Tainter  
5:00 Phenotypic Segregation (Herbicide Tolerance) Analysis in Elite Inbred Transgenic Maize  
P-1011 P. BULLOCK, M. Galatowitsch, D. Foster, K. Newhouse, C. Lewnau, K. Cook, S. Bagnall, J. Chojecki, V. Guerin, M. Wilson, S. Jiao, J. Register, I. Evans, K. Wang, M. Alphas, T. Friend  
5:15 Reduction of Ionic Mercury by Transgenic Plants  
P-1012 H.D. WILDE, N.M. Stack, L.V. Azarraga, and R.B. Meagher

4:30 pm - 6:00pm

POSTER PRESENTATION

Cellular Toxicology Poster Authors Will Be Present  
Monday, June 6

T-1001 to T-1036 (See List of Posters on Pages ???-???)

MONDAY, JUNE 6

7:00 pm - 9:00 pm

WORKSHOP

Auditorium

*Co-Sponsored by the Baker Company, LabConco Corporation, and Marily H. Elam, Esq.  
Hosted by the Laboratory Materials and Biosafety Committee, Toxicology Committee,  
Cell Standardization Committee, and the Vertebrate Division*

**T Law and Liability in the Laboratory**

*Co-Conveners: Sandra L. Schneider, The University of Texas Health Science Center, and  
Errol Zeiger, National Institute of Environmental Health Sciences*

There is a need for the laboratory professional to understand the basic legal and liability issues in safety management, and understand torts relative to professional liability. Regardless of whether laboratories are engaged in routine testing, basic research, or product development, for profit or not-for-profit, they are workplaces subject to the full range of laws governing liability, safety compliance, employment practice, and business dealings. In addition, there are safety and performance standards that have been enacted or proposed specifically for laboratories. This session will address legal issues affecting laboratories and laboratory personnel in general terms, with emphasis on conflict of interest and research misconduct issues. The panel will include three lawyers with backgrounds in science, academe, and business who will welcome discussion with the audience.

- |      |   |
|------|---|
| 7:00 | Overview of Current Legal Issue Relevant to the Scientist and Laboratory<br>E. ZEIGER |
| 7:30 | Liability Concerns for the Researcher and Laboratory Personnel<br>D. WARREN           |
| 8:00 | Business Law and Conflict-of-Interest Problems for the Laboratory<br>M.H. ELAM        |
| 8:30 | Open Forum  |

7:00 pm - 9:00 pm

WORKSHOP

Royal Ballroom

**I A Feeling for the Organism: Novel Approaches to the Culture of Difficult Invertebrate Cells**

*Convener: Timothy J. Kurtti, University of Minnesota*

7:00 pm - 9:00 pm

WORKSHOP

Empire C&D

**T BioCoat® Cell Environments: Construction of In Vitro Models for Hepatocytes and Enterocytes**

*Convener: Brigitta Tadmor, Collaborative Biomedical Products*

**Rapid Formation of a Differentiated Monolayer of Caco-2 Cells With Barrier Function**  
M. SWIDEREK, Collaborative Biomedical Products

A cell culture system optimized for the rapid development of barrier function in cultured intestinal epithelial cells is presented. Barrier function has been measured by transepithelial electrical resistance, and mannitol or rifampin permeability.

**Maintenance of Liver-Specific Functions In Hepatocytes Cultured Over Extended Periods of Time**

B. TADMOR, Collaborative Biomedical Products

The importance of the extracellular matrix as part of a cell culture system that allows for maintenance of differentiated hepatocytes *in vitro* is discussed. The degree of hepatocyte differentiation has been determined at the level of cell morphology and function.

**MONDAY, JUNE 6**

**Factors Regulating Gene Expression in Primary Rat Hepatocytes**

J. SIDHU, University of Washington

The effect of cell culture conditions on the expression of cytochrome P450 (CYP genes) and liver-specific genes is discussed focusing on the role of various configurations and types of extracellular matrix components.



## TUESDAY, JUNE 7

10:00 am **COFFEE BREAK** Exhibit Hall

10:00 am-5:00 pm **EXHIBITS AND POSTERS** Exhibit Hall  
*Posters must be removed from Exhibit hall  
 by 5:00 pm Tuesday, June 7*

10:30 am-12:00 pm **POSTER PRESENTATION**  
 Plant Poster Authors will be present  
 Tuesday, June 7  
 P-1021 to P-1084 (*See List of Posters on Pages 22-24*)

10:30 am - 12:30 pm **SESSION-IN-DEPTH** Imperial VI-VII  
*(See Abstracts on Pages 43-44)*  
*Sponsored by Johnson and Johnson*

**T The Role of Cell Mediators in the Toxic Response**  
*Convener: Jaspreet S. Sidhu, University of Washington*

This Session-In-Depth will focus predominantly on the modulation of toxic challenges to cells by secondary intracellular mediators and by extra cellular agents modulating the latter's activity.

10:30 cAMP-Mediated Inhibition of Phenobarbital-Inducible CYP2B1, CYP2B2, and CYP3A1 Gene  
 Expression in Primary Rat Hepatocyte Cultures  
 T-21 J.S. SIDHU and C.J. Omiecinski  
 11:00 Mechanisms of Activation of Neutrophils and Neutrophil-Mediated Toxicity  
 T-22 P.E. GANEY  
 11:30 The Effects of Toxicant-Induced Changes in Thiol Status on Transmembrane Signal  
 Transduction in Human and Mouse T-lymphocyte Subsets  
 T-23 T.J. KAVANAGH, R.A. Ponce, A.J. Potter, D.L. Eaton, P.S. Rabinovitch, and A. Grossman  
 12:00 Expression of Class Alpha, Mu and Pi Glutathione S-transferases and Cytochromes  
 P4501A1, 1A2, 2B1, 2B2, and 2E1 in Rat and Rabbit Primary Cultured Hepatocytes  
 T-24 R.F. NOVAK, R. Dwivedi, R. Zangar, and A. Gruebele

10:30 am - 12:30 pm **CONTRIBUTED PAPERS** Empire C&D  
*(See Abstracts on Pages 97-98)*

**V Differentiation and its Modulation**  
*Convener: Meg Schelling, Washington State University*

10:30 Regulation of Ciliated Cell Differentiation in Cultures of Rat Tracheal Epithelial Cells  
 V-1012 A.B. CLARK, T.E. Gray, T. Bader, P. Nettesheim, and L.E. Ostrowski  
 10:45 Modulation of Angiogenesis by Anti-Fibroblast Growth Factor Monoclonal Antibody  
 V-1013 M.E. Schelling, S. Venkateswaran, V. Blanckaert, and A. Zijlstra  
 11:00 Transplantation of a Bioengineered Bone Marrow Tissue and Its Potential Use as a Sub-  
 strate for Toxicity Assessment  
 V-1014 B.A. NAUGHTON, J. San Román, B. Sibanda, J. Gee, D. Morales, and V. Kamali  
 11:15 Effects of Trypsin on the Surface Architecture of Embryonic Spinal Ganglion Cells *In Vitro*  
 V-1015 E. Lindner, M. Inczedy-Marcsek, L. HSU

**TUESDAY, JUNE 7**

- |       |        |   |
|-------|--------|---|
| 11:30 |        | Culture of Neuronal Cells from Zebrafish Embryos  |
|       | V-1016 | C. GHOSH and P. Collodi   |
| 11:45 |        | The <i>de novo</i> Activation of the Vitellogenin Gene Family is Accompanied by a Spatial |
|       |        | Rearrangement of Centromeric Domains  |
|       | V-1017 | J. JANEVSKI, P.C. Park, and U. De Boni  |

2:30 pm - 5:00 pm

**SESSION-IN-DEPTH**  
(See Abstracts on Pages 48-50)

Imperial VI-VII

**V Multicellular Organization and Differentiation in Culture**

*Convener:* Joseph Leighton, Aeron Biotechnology

On microscopic examination of epithelial tissues in nature, normal or cancerous, it is evident immediately that proliferating cells are not evenly distributed throughout individual assemblies of cells. To simulate and recognize comparable spatial organization in culture requires three-dimensional growth and other essentials as well. The breast, skin, and trachea in histotypic cultures are the tissues to be considered here. In addition, collagen waffle membranes in gradient culture will be described.

- |      |      |  |
|------|------|--|
| 2:30 |      | Introduction (J. Leighton)   |
| 2:35 |      | Multicellular Reorganization by Single Cells on Extracellular Matrix                             |
|      | V-13 | M.H. BARCELLOS-HOFF  |
| 2:50 |      | Stromal Interactions in Growth and Adhesion of Breast Carcinoma Cells: Role of Growth            |
|      |      | Factors and Extracellular Matrix   |
|      | V-14 | B.E. ELLIOTT, B. Bhardwaj, R. Lall, D. Leopold, N. Rahimi, R. Saulnier, M. Park, and T. Nakamura |

**3:05 DISCUSSION**

- |      |      |   |
|------|------|---|
| 3:15 |      | Progress in Studying the Growth and Cycling of Hair Follicles                             |
|      | V-15 | A.E. BUHL, D.J. Waldon, T.T. Kawabe, and K.E. Kappenman                                   |
| 3:30 |      | Liposome Targeting of Functional DNA to the Hair Follicles of Histocultured Skin: A Model |
|      |      | for Gene Therapy of Hair Growth   |
|      | V-16 | L. Li, V. Lishko, and R.M. HOFFMAN  |

**3:45 DISCUSSION**

- |      |      |   |
|------|------|---|
| 3:55 |      | Modulation of Differentiation of Rat Tracheal Epithelial (RTE) Cells by Exogenous Matrix in |
|      |      | Air-Liquid Interface Cultures   |
|      | V-17 | E.A. Davenport and P. NETTESHEIM  |
| 4:10 |      | Regulation of Tracheobronchial Mucous Cell Differentiation in Culture                       |
|      | V-18 | R. WU   |

**4:25 DISCUSSION**

- |      |      |   |
|------|------|---|
| 4:35 |      | Collagen Membrane Waffle Culture: Defining the Place of Growth Modulators in Histokinetic |
|      |      | Processes   |
|      | V-19 | J. LEIGHTON   |

**4:50 DISCUSSION**

## TUESDAY, JUNE 7

2:30 pm - 4:45 pm

SESSION-IN-DEPTH  
(See Abstracts on Pages 36-37)

Empire ABC

**P In Vitro Plant Resources for Valuable Products***Convener:* Mary Ann Lila Smith, University of Illinois, and Paul Read,  
University of Nebraska

Plants, and plant cells in vitro, are indisputably superior organic chemists. But how well have we capitalized on their potential to produce fine chemicals for human use? J. Fitchen will report on the use of plant cells as microbial system-style factories to produce products that are *not* typical plant metabolites (e.g. vaccines, antibodies). He will also draw the parallel between in vitro systems for secondary metabolite production and non-plant products. P. Weathers will overview the range of production systems and bioreactor types used to cultivate a diversity of plant cell species and products. M.A.L. Smith will detail progress with a specific in vitro target group - anthocyanin-rich plants, and R. Shillo reports on analysis of valuable alkaloids in both in vivo bulbs and in vitro tissues. K. Kadkade will focus on industrial-scale utilization of plant cell cultures, especially for pharmaceutical products.

2:30	Introduction (M.A.L. Smith)
2:35	Production of Monoclonal Antibodies and Candidate Vaccines in Plants and Plant Cell Cultures
P-17	J.H. FITCHEN
3:05	Systems for Production of Secondary Metabolites from Plants In Vitro
P-18	P.J. WEATHERS
3:35	Natural Pigments and Medicinal Compounds from Anthocyanin-rich Plant Cells
P-19	M.A.L. SMITH and D.L. Madhavi
3:55	Assay of Alkaloids in Narcissus and Other Amaryllidaceae by HPTLC and HPLC
P-20	R.D. SHILLITO, I. Rouse, and N. Andersen
4:15	Large-Scale Production of Secondary Metabolites from Plant-Cell Cultures
P-21	P.G. KADKADE and E.J. Kane

2:30 pm - 4:30 pm

SESSION-IN-DEPTH  
(See Abstracts on Pages 42-43)  
*Sponsored by Clonetics Corporation*

Imperial VI-VII

**T In Vitro Test Systems in Toxicology***Convener:* Mary Taub, Roswell Park Memorial Institute, and Richard Ham, University of Colorado

2:30	2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Stimulation of Xenobiotic Toxicity in a Spontaneously Immortalized Line of Human Epidermal Keratinocytes
T-17	R.H. RICE, A.A. Walsh, and L.A. deGraffenried
3:00	Developing Better Defined Media for Toxicity Testing with Normal Human Cells
T-18	R.G. HAM
3:30	Toward an <i>In Vitro</i> System for Testing Xenobiotic Effects on Mammary Function
T-19	M.C. NEVILLE
4:00	Immortalization of Rabbit Kidney Proximal Tubule Cells in Serum-Free Medium
T-20	M. TAUB, C. Allen, H.J. Han, and J.H. Park

**ATTENTION POSTER PRESENTERS**  
*All Posters Must Be Removed From Exhibit Hall*  
*By 5:00 p.m. Tuesday, June 7*

BANQUET 7:00 pm - 10:00 pm

*Seating is Limited*  
*Admittance to Banquet by Advance*  
*Ticket Holders Only*

**SUNDAY, JUNE 5**  
10:00 am to 6:00 pm

**MONDAY, JUNE 6**  
10:00 am to 6:00 pm

**TUESDAY, JUNE 7**  
10:00 am to 5:00 pm

**POSTER SESSION**

*Posters Mounted Saturday, June 4, 3:00 pm*  
*Poster Must Be Removed From Exhibit Hall By 5:00 pm, Tuesday, June 7*

Authors Will Be Present At Their Posters The Following Days and Times:

**Sunday, June 5**  
Vertebrate and Invertebrate  
4:30 pm - 6:00 pm

**Monday, June 6**  
Cellular Toxicology  
4:30 pm - 6:00 pm

**Tuesday, June 7**  
Plant  
10:30 am - 12:00 pm

**CELL-CELL INTERACTIONS**

- V-1018 Coculture of Epithelial and Stromal Endometrial Cells Provides Model for Study of Cell-Cell Interactions in Neoplasia. J.T. ARNOLD, J.F.H. Blasser, D.G. Kaufman.
- V-1019 Effect of Dichloroacetic Acid, Trichloroacetic Acid and Chloral Hydrate on Intercellular Communication in Clone 9 Rat Hepatocyte Cells. S.G. BENANE, C.F. Blackman, and D.E. House.
- V-1020 Growth of Microvascular Retinal Cells in Serum-Free Media. I.M. EVANS, L.F. DeTulleo, H.L. Huyck, S.J. Leuenroth, J.E. Maul, C.M. Stocum, and J.F. Walter.
- V-1021 Ion Transport Across a High-Resistance Blood-Brain Barrier Examined by Non-Invasive Voltage Probes. P.J.S. SMITH and A.M. Shipley.
- V-1022  $\beta_2$ -Microglobulin-Dependent Positive Co-Operation Between Antigen-Presenting Cells. U. ARMATO, J. Wu, and D. Barisoni.

**BIOTECHNOLOGY**

- V-1023 Non-Invasive Voltage Probe (NVP) for the Measurement of Steady Ionic Currents. J.G. Kunkel, A.M. Shipley, R.H. Sanger, and P.J.S. SMITH.
- V-1024 Entrapment of Hepatocyte Spheroids in a Hollow Fiber Bioartificial Liver for Potential Treatment of Liver Failure. F.J. WU, M.V. Peshwa, F.B. Cerra, and W-S. Hu.
- V-1025 A Stereotypic, Transplantable Liver Culture System. B.A. NAUGHTON, B. Sibanda, J. San Román, J. Gee, D. Morales, and V. Kamali.
- V-1026 New Scale-Up Technologies for Anchorage-Dependent Cell Culture. R.W. Boice and A.J. Meuse.
- V-1027 Production of Human Recombinant Pro-Stromelysin I in a Baculovirus Expression System. P.R. BENTON, M. Walroth, P. Cannon, and P. Belloni.
- V-1028 The Hybridoma Bank: Hybridoma Cell Lines Available for Basic Research. K. STEENBERGEN and P.R. McClintock.
- V-1029 Trends in the Incidence and Distribution of Mycoplasma Contamination Detected in Cell Lines and Their Products. V. Pawar, J. Luczak, M.S. Cox, J. Dubose, Jr., and J.W. HARBELL.

**CANCER BIOLOGY**

- V-1030 Characterization of Clones from *Ras* (TG-AC) Transgenic Mouse Skin Tumors. J-L.D. Klein, C. Szczesniack, R. Cannon, C. Trempus, J. Spalding, and J. Roberts.
- V-1031 Visualizing the Molecular Dynamics of the Actin-Cytoskeleton in Normal and Transformed Cells Using Light-Optical-Based Reagents. K.A. GIULIANO and P.L. Kornblith.
- V-1032 Development of An Ulcerative Colitis-Derived Cell Line. G.C. BALCH, L.A. Manzano, J.S. Stauffer, and M.P. Moyer.
- V-1033 Development and Characterization of Human Normal and Cancerous Stomach Epithelial Cell Lines. L.A. MANZANO, G.C. Balch, J.S. Stauffer, and M.P. Moyer.
- V-1034 Development and Characterization of Two Novel Human Colon Polyp (COP) Cell Lines with Differing Degrees of Tumorigenicity. J.S. STAUFFER, L.A. Manzano, G.C. Balch, R.L. Merriman, L.L. Tanzer, and M.P. Moyer.

**GENERAL SUBJECTS—INVERTEBRATE/VERTEBRATE-RELATED**

- V-1035 Bacterial L-Forms Are Omnipresent in "Sterile" Tissue Culture Sera. J.W. HAYCOCK, K.E. Joho, J.C. Galyon, and R.L. Moses.
- V-1036 Isolation and Characterization of Functional Dendritic Cells in Rat Choroid. A. CHOUDHURY, V.A. Pakalnis, and W.E. Bowers.
- V-1037 Production and Characterization of a Monoclonal Antibody Specific to *Mycobacterium tuberculosis*. J. KAPUR, S.K. Gupta, and G.P. Talwar.
- V-1038 Role of Interferon on Influenza Induced Genotoxicity. M.V. RAMANA and G. Sharma.
- V-1039 An Efficient Method for Routine Epstein-Barr Virus Immortalization of Human B Lymphocytes. F.E. WALL, R.D. Henkel, M.P. Stern, and M.P. Moyer.
- V-1040 Effect of Serum-Free Media and Extracellular Matrix on the Growth and Gene Expression of OR-HEPA B<sub>3</sub> Cells. M. VEGA, N. Cordero, and M. Morales.
- V-1041 Effects of Interstitial ECM on the Viability of Human Neutrophils *In Vitro*. E.J. ROEMER, K.J. Stanton, and S.R. Simon.
- V-1042 Differentiation of Stem Cells from the Midgut of the Insect, *Manduca sexta*, Occurs *In Vitro* in the Presence of a Factor From Mature Larval Midgut Cells. S. Sadrud-Din, R.S. Hakim, and M.J. LOEB.

**DIFFERENTIATED CELLS**

- V-1043 An Organ Culture Model of the Embryonic Murine Ear. D.S. HOFFMAN, P. Bringas, and H.C. Slavkin.
- V-1044 Properties of the Hepatix C3A Human Hepatoblastoma Cells Cultured in a Hollow Fiber Bioreactor. A. SPIERING, D. He, M. Harrison, Y. W. Lo, A. Rotem, N.L. Sussman, and J.H. Kelly.
- V-1045 Selective Expression of Prostaglandin H Synthase-1 and -2 During Differentiation of Rat Tracheal Epithelial Cells. E.M. Hill, T. BADER, P. Nettesheim, and T.E. Eling.
- V-1046 Regulation of Growth and Secretory Differentiation in Cultured, Normal, Early Passaged Human Bronchial Epithelial (HBE) Cells. T. GRAY, T. Bader, and P. Nettesheim.
- V-1047 Regulation of Mucin Expressions by Retinoic Acid (RA) and Culture Conditions in Rat Tracheal Epithelial (RTE) Cells. V.B. GODFREY, K. Guzman, C.B. Basbaum, P. Nettesheim, and S.H. Randell.
- V-1048 Flow Cytometric Analysis of Mouse Bone Marrow. G.D. KALMAZ.

**GROWTH AND SYNTHESIS**

- V-1049 Pyridoxine HCl: An Equivalent Form of Vitamin B-6 for Dulbecco's-Modified Eagle's (DME) Cell Culture Medium. B. ALDERETE, L. Balog, J. Doak, K. Etchberger, R. Festen, and J. Keathely.
- V-1050 Growth of Myogenic Cells in Media Supplemented With Peptide-Bound Essential Amino Acids. Y.L. PAN, K.E. Webb, Jr., and P.K. Bender.
- V-1051 The Effect of Secretory Factors of a Hepatoma Cell Line As Growth Regulators. Y. ORTIZ, N. Cordero, and M. Morales.
- V-1052 Utilization of Peptide-Bound Methionine for the Synthesis of Secreted Proteins by Cultured Bovine Mammary Epithelial Cells. S. WANG, K.E. Webb, Jr., and R.M. Akers.

**CELLULAR MODELS**

- V-1053 Three-Dimensional Culture of Bovine Chondrocytes in Rotating-Wall Vessels. T.L. Prewett and T.J. Goodwin.
- V-1054 Isolation, Purification and Quantitation of Mast Cells from Rat Uterus. V. CHOPRA and R.E. Garfield.
- V-1055 The Culture of Chick Embryo Ovary as a Model in Experimental Tumorigenesis. R. AVILA, M. Samar, S. de Fabro, and R. Ferraris.
- V-1056 A Method for the Culture of Proliferating Sheets of Sensory Cell Progenitors Isolated from the Chick's Cochlea. J.E. FINLEY and J.T. Corwin.
- V-1057 Prohibitin Expression in Immortalized Cell Populations. R.T. DELL'ORCO, X-T. Liu, E.R. Jupe, J.L. Kiehlbauch, and J.K. McClung.
- V-1058 Normal Endothelial Cell Proliferation and Control Using a Novel Culture Fluid. V. CALDWELL, V. Mayer, R. Wilkinson, and A.R. Torres.

SILENT

- V-1059 E1A-NR.1: An E1A-Immortalized Retinal Cell Culture with Serum-Sensitive Glial/Neuronal Properties. G.M. SEIGEL, F.Q. Liang, and M. del Cerro.
- V-1060 Primary Culture of Carp, *Cyprinus carpio*, Hepatocytes. R. Böhm and H. Segner.
- V-1061 Mycoplasma Testing of Cell Cultures by a Combination of Direct Culture and DNA-Fluorochrome Staining. D.J. Lundin and C.K. Lincoln.
- V-1062 Antibiotic Resistance of Mycoplasma Isolates from Cell Cultures. C.K. Lincoln and D.J. Lundin.
- V-1063 Jumbocytes in Mitosis, A Putative Source of Genetic Heterogeneity. J. Leighton.
- V-1064 Cells on Fibers (Rotated and Corrugated). R. CLYDE.
- V-1065 Inhibitory Effect of Albendazole on Succinate Dehydrogenase of Tape Worm (*Neokrimia Singhia*). M.R. SIVA SAI KUMARI and R. Rao.

# PLANT POSTERS

**SUNDAY, JUNE 5**  
10:00 am to 6:00 pm

**MONDAY, JUNE 6**  
10:00 am to 6:00 pm

**TUESDAY, JUNE 7**  
10:00 am to 5:00 pm

## POSTER SESSION

*Posters Mounted Saturday, June 4, 3:00 pm*  
*Poster Must Be Removed From Exhibit Hall By 5:00 pm, Tuesday, June 7*

Authors Will Be Present At Their Posters The Following Days and Times:

**Sunday, June 5**  
Vertebrate and Invertebrate  
4:30 pm - 6:00 pm

**Monday, June 6**  
Cellular Toxicology  
4:30 pm - 6:00 pm

**Tuesday, June 7**  
Plant  
10:30 am - 12:00 pm

### PLANT REGENERATION

- P-1021 Regeneration of Sunflower (*Helianthus annuus* L.) From Mature Cotyledons. C. BAKER, N. Fernández and C. Carter.
- P-1022 Tetraphenylboron and Phenylboronic Acid Induce Somatic Embryogenesis. J. Ponsamuel and P. Dayanandan.
- P-1023 Thidiazuron Mediated *In Vitro* Regeneration of Peanut Plants. M. KANYAND, A. Porobo Dessai, and C.S. Prakash.
- P-1024 In Vitro Systems for Plant Regeneration of Switchgrass. K. ALEXANDROVA, P.D. Denchev, and B.V. Conger.
- P-1025 Somatic Embryogenesis In Rapid Cycling (*Brassica rapa*). R. BANKS, M.R. Uddin, G. Small, and M.I. Shafi.
- P-1026 A Rapid and Repetitive Somatic Embryogenesis System in Sweetpotato. Q. Zheng, A. Porobo Dessai, and C.S. Prakash.
- P-1027 Cryopreservation of Embryonic Axes of Hazelnut (*Corylus avellana* L. cv. Barcelona). B.M. REED and M.N. Normah.
- P-1028 Anther Culture Studies in Pigeonpea. P. VIJAYAKUMARI and S. Narasimha Chary.
- P-1029 Somatic Embryogenesis and Plant Regeneration in Chickpea (*Cicer arietinum* L.). A.K. Sudha Vani and V.D. Reddy.
- P-1030 Induction of Somatic Embryos in Suspension Cultures and Plant Regeneration of Indica Rice. T. USHA RANI and G.M. REDDY.
- P-1031 In Vitro Development of An Interspecific (*O. Sativax O. Longistaminata*) Hybrid and Cytological Status of Regenerated Hybrid Plant. N. FATIMA, S.Y. Anwar, and T.P. Reddy.
- P-1032 Plantlet Regeneration-Influence of Genotype, Explant Age and Hormones on Rhizogenesis in Safflower (*Carthamus tinctorius* L.). T. GUDIPATI and R.R. Das.
- P-1033 Regeneration of Cucumber (*Cucumis sativus* L.) Plants From Excised Cotyledon. A.K. MISRA and S.P. Bhatnagar.
- P-1034 Occurrence of Developmental Abnormalities in Callus Cultures of *Eruca sativa*. N. JOHN and A. Batra.

### TRANSFORMATION

- P-1035 Transfer and Expression of T-DNA into Rice and Sorghum via *Agrobacterium*. R.H. SMITH, T.S. Ko, and S.H. Park.
- P-1036 Development of Herbicide Resistant Turfgrass Through Mutant Selection and Protoplast Transformation. L. LEE, C. Laramore, P. Day, and N. Tumer.
- P-1037 Transformation of Caucasian Bluestem (*Bothriocloa caucasica*, L.). J. Ponsamuel, A. Trieu, D.V. Huhman, and C.J. Franklin.
- P-1038 Rapid Production of Transgenic Corn By Microprojectile Bombardment of High Type II Immature Embryos. A.S. Wang, E. Brambila, and R.A. EVANS.
- P-1039 Optimization of PEG-Mediated Stable Transformation for Regenerable Maize Protoplast Cultures. A.S. WANG, R.A. Evans, and J.L. Rosichan.
- P-1040 Transient GUS Expression In Lily Bulb Scales and Cell Suspensions Transformed By Particle Bombardment. J.M. PEÑAFIEL and K. Kamo.
- P-1041 Effects of Concentration of Acetosyringone and *Agrobacterium tumefaciens* on GUS Gene Transformation Efficiency of *Populus*. F.H. HUANG and X.Y. LI.
- P-1042 Stable Transformation of Long Day Photoperiod-Adapted Soybean Somatic Embryogenic Culture. L.-N. TIAN, D.C.W. Brown, H. Voldeng, and J. Webb.

- P-1043 The Role of DNA Methylation in the Expression of the Dc8-GUS Transgene in Carrot (*Daucus carota* L.). Y. ZHOU, J.M. Magill, C.W. Magill, and R.J. Newton.
- P-1044 Somatic Hybridization of *Sinapis alba* and Rapid Cycling *Brassica oleracea*: A Step Toward Transfer of Pest Resistance Into *Brassica* Vegetables. L.N. HANSEN and E.D. Earle.
- P-1045 A Simple, Reliable Transformation Method for *Brassica oleracea*. T.D. METZ, R. Dixit, and E.D. Earle.
- P-1046 The Effects of Seedling Age and Light Intensity on *Brassica napus* L. C. LI and J.C. Turner.
- P-1047 Regeneration and Transformation of *Arachis* spp. M. Cheng, Z. Li, A. Xing, R.L. Jarret, R.N. Pittman, and J.W. Demski.
- P-1048 Co-Transformation and Subsequent Segregation Frequencies in Tobacco Utilizing an *Agrobacterium* Strain Containing Two Binary Plasmids. M. DALEY and V. Knauf.
- P-1049 Effect of Overexpression of SOD on Growth of Transgenic Plants Under Drought and Low Temperature. N.L. TROLINDER and R.D. Allen.
- P-1050 Development of Marker System in Wild *Nicotiana* Species Via Direct Gene Transformation. V. Ilcheva and M. Vlahova.
- P-1051 A Glyphosate-Tolerant EPSP Synthase Gene as a Selectable Marker in Wheat Transformation. H. ZHOU, J.E. Fry, J.W. Arrowsmith, T.W. Corbin, and M.E. Fromm.

**MICROPROPAGATION**

- P-1052 Bulblet Formation From Three Varieties of *Allium sativum* *In Vitro*. M.L. CARDENAS, T.E. Torres, R. Mercado, J.A. Villarreal, and E. Cárdenas.
- P-1053 Micropropagation From Shoot Tips of Tomato (*Lycopersicon esculentum* [Mill]). J. AMBRIZ, E. Olivares, E. Cárdenas, and F. Montes.
- P-1054 Automated Sucrose Concentration Control in Micropropagation Media. S.A. Hale and R.E. Young.
- P-1055 *In Vitro* Clonal Propagation of Country Potato—An Under-Exploited Tuber Plant. J. Ponsamuel, N.P. Samson, and K.P. Anderson.
- P-1056 Cotton Fiber as a Substitute for Agar Used in Plant Tissue Culture. R.M. CERDEIRA, J.V. Krans, J.D. McChesney, A.M.S. Pereira, and S.C. Franca.
- P-1057 The Effect of BA and NAA in *Podophyllum* Shoot and Rhizome Culture. R.M. CERDEIRA, C. Burandt, Jr., and J.D. McChesney.
- P-1058 Factors Affecting High Frequency Shoot Regeneration From Leaf Explants of Muskmelon; Enhancement by the Sulfonylurea Herbicide, Pinnacle. R.C. YADAV, M. Saleh, and R. Grumet.
- P-1059 Behavior of Rhododendron Tissue Affected by Tissue Proliferation. M.H. BRAND and R. Kiyomoto.
- P-1060 Micropropagation of a Dwarf Trumpet Vine, *Campsis* sp. W.A MACKAY.
- P-1061 Influence of Thidiazuron, Gibberellic Acid, and Abscisic Acid on *In Vitro* Shoot Formation from Excised Roots of Silktree. T.D. DAVIS and N. Sankhla.
- P-1062 Micropropagation of Indica Rice Through Proliferation of Axillary Shoots. J.S. SANDHU, S.S. Gosal, M.S. Gill, and H.S. Dhaliwal.
- P-1063 Somaclonal Variation in Tissue Culture Derived Plants of Pigeonpea. CH. PRASANALATHA, J.P. Moss, K.K. Sharma, and J.K. Bhalla.
- P-1064 Micropropagation of Pomegranate Jyoti from Axillary Bud Explants. A. MURALIKRISHNA and A.N.S. GOWDA.

**DEVELOPMENTAL AND CELL BIOLOGY**

- P-1065 The Effects of Methionine Sulfoximine on Protein and RNA Synthesis of Soybean. E.L. MYLES, M. Zheng, and C. Caudle.
- P-1066 The Effects of Atrazine on Protein Synthesis in Soybean Callus. P.S. KAHLON and S.M. Bhatti.
- P-1067 Genotype Specific Polypeptide Induction With Abscisic Acid and Water Stress in Callus Cultures of Rice. K. UMA RANI and G.M. REDDY.
- P-1068 Effects of Cold Preincubation, Aminoxyacetic Acid and Cobalt on Ethylene Emanation and Somatic Embryogenesis from Orchardgrass Leaf Cultures. A.I. KUKLIN, Z. Tomaszewski, Jr., C.E. Sams, and B.V. Conger.
- P-1069 *In Vitro* Selection for Salt Tolerance in Cultivars of Indica Rice. J. Ponsamuel and E.W. Weiler.
- P-1070 Influence of Cd and Pb on Growth, Proline Level in Calli of Two Species *Nicotiana*. P. Nikolova, J. Topalova, S. Chankova, and G. Nickoff.

## PLANT POSTERS

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- P-1071 Carbon and Nitrogen Utilization By Soybean Seeds Grown In Vitro. C.H. Saravitz and C.D. Raper, Jr.
- P-1072 Antioxidant Levels and Activities of Related Enzymes in Carnation Plantlets During Thidiazuron-Induced In Vitro Hyperhydricity. D. Sankhla, S. Trivedi, T.D. DAVIS, and N. Sankhla.
- P-1073 Microscopical Observation of Culture Tissues of Plant. Y. TAHAMA.

### PLANT IMPROVEMENT

- P-1074 Use of Tissue Culture Techniques to Improve Louisiana Soybeans. S.S. CROUGHAN.
- P-1075 Use of Somatic Embryogenesis for Hybrid Alfalfa Seed Production. A.R. McElroy and L.J. LANGILLE.
- P-1076 *In Vitro* Screening for Ascochyta Blight Resistance in Chickpea (*Cicer arietum* L.). A.K. Sudha Vani and V.D. Reddy.
- P-1077 Screening of Four Rice Cultivars at Cellular Level Against *Xanthomonas oryzae* pv. *oryzae*. R. KAUR, B.S. Thind, S.S. Gosal, and J.N. Gupta.
- P-1078 Production of Thermostable Amylolytic Enzymes From *Clostridium* sp. M.V. SWAMY and G. Seenayya.

### PLANT SECONDARY METABOLISM

- P-1079 Effect of Hormones on Callus of Mikania sp. A. CERDEIRA, E. Gardini, A. Pereira, and S. França.
- P-1080 Effect of Auxins and Cytokinins on Anthocyanin Production in Cranberry Cell Cultures. D.L. MADHAVI and M.A.L. Smith.
- P-1081 Thin Cell Layer Culture of a Monocot, *Setcreasea pallida* (syn. *Tradescantia pallida*) cv. Purple Heart. D.W. DARNOWSKI and E.D. Earle.
- P-1082 Theanine Formation by Tea Cells. T. Takihara, I. Sakane, and T. KAKUDA.
- P-1083 Metabolism of Carbofuran in Cell Suspension Cultures of Sugarcane as a Function of Concentration and Incubation Time. S. KAUR, B. Singh, S.S. Gosal, and R.L. Kalra.
- P-1084 Morphogenetical Regulation Under the Influence of Phytohormones in *Cuminum cyminum*. A. DAVE and A. Batra.
- P-1015 Optimization of Silicon Carbide Fiber-Mediated DNA Delivery Into Regenerable Sorghum and Maize Tissue Cultures. H.F. KAEPLER, J.F. Pedersen, and D.A. Somers.

### SILENT

- P-1085 Rapid Transformation of Eggplant (*Solanum melongena* L.) Using *A. tumefaciens* Carrying a Binary Vector Containing the Coat Protein Gene From Cucumber Mosaic Virus (CMV). J.F. REYNOLDS, A. Wilkerson, D. Tricoli, P. Russell, H. Quemada, R. McMaster, S. Mastenbrook, and L. Herrygers.
- P-1086 In Vitro Tumor Formation on *Psidium guajava* Infected by *Agrobacterium tumefaciens*. O. VAZQUEZ-MARTINEZ, J.L. Moreno-Hernandez, and L.L. Valera-Montero.
- P-1087 Genetic Transformation and Transgenic Plant Regeneration of Grapevine. L. MARTINELLI and G. Mandolino.
- P-1088 Bio-Technological Aspects of In Vitro Multiplication of Certain Biomass Yielding Plants. CH. AYODHYA RAMULU and D. Rao.
- P-1089 Somatic Embryogenesis and Plant Regeneration in Cassava (*Manihot esculenta* Crantz.). T.C. NARAYANASWAMY, N.M. Ramaswamy, and S.R. Sree Rangaswamy.
- P-1090 Anther Culture Response of Wheat and Wheat x Wheatgrass Hybrids. H.C. Sharma, O. Benlhabib, H.W. Ohm, and C.S. Lu.
- P-1091 Efficient Plant Regeneration from Callus Cultures of Mulberry Trees. Y. SAHOO, S.K. Patnaik, and P.K. Chand.
- P-1092 Regeneration Studies in Pigeonpea Varieties of Different Maturity Groups. P. KAUR and J.K. Bhalla.
- P-1093 Cassava Root Thickening *In Vitro*. D.W. DARNOWSKI and E.D. Earle.
- P-1094 Different Embryogenic Competence Within Single Explants of *Camellia Japonica*. M.C. PEDROSO and M.S. Pais.
- P-1095 Ureide Metabolic Pathway in  $N_2$  Fixing Pigeonpea (*Cajanus cajan* L.). D. SREENIVAS REDDY, G. Reddy, and H. Polasa.
- P-1096 Rapid Micropropagation of *Dalbergia sissoo* Roxb. V.A. Chauhan, P.C. Josekutty, and G. Prathapasenan.
- P-1097 Cloning of 20-Year-Old Female Jojoba (*Simmondsia chinensis* [Link.] Schneider). R.P. SINGH, T.S. Rathore, S. Rama Rao, and N.S. Shekhawat.

## PLANT POSTERS

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- P-1098 Rapid Micropropagation of *Ficus religiosa* L. S.R. Deshpande, P.C. Josekutty, G. Prathapasenan.
- P-1099 Micropropagation of Grapes (*Vitis vinifera* L.) cv. Thompson Seedless. A.N.S. GOWDA and A. MURALIKRISHNA.
- P-1100 Micropropagation Strategy for Guava (*Psidium guajava* L.) cv. Sardar. A.N.S. GOWDA and A. MURALIKRISHNA.
- P-1101 Breeding Tomato Plants for Regenerability In Vitro. N.M. Piven, M. Sánchez, D. Infante.
- P-1102 Influence of Culture Medium Strength and Growth Regulators on the Micropropagation of *Dioscorea yams*. S.A. MITCHELL and H.N. Asemota.

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Plant  
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**MODEL DEVELOPMENT**

- T-1001 Development of an In Vitro Canine Blood Brain Barrier Models. T.R. PIPPERT, D.R. Umbenhauer, W.W. Nichols.
- T-1002 Development of Mammalian Cell Lines Stably Expressing Mouse Prostaglandin Synthase 1 and 2. P.C. CHULADA, V.D. Winn, D.A Young, H.F. Tiano, K.R. Tindall, C.D. Loftin, T.E. Eling, and R. Langenbach.
- T-1003 *In Vitro* Fertilization Enhances the Sensitivity and Utility of the FETAX Assay. R. VARNOLD, E. Elmore, and L.D. Smith.
- T-1004 Initial Characterization of a New Model for Ocular Irritancy Testing. H.A. Ricker, P.J. Neal, J. Kubilus, and M. KLAUSNER.

**KERATINOCYTE SYSTEMS**

- T-1005 Cellular Differentiation Is Enhanced in a Proliferating Culture of Epidermal Keratinocytes Exposed to Bis-( $\alpha$ -chloroethyl)-sulfide (BCES). L. BERNSTAM, A. Kotlyar, F.L. Vaughan, and I.A. Bernstein.
- T-1006 Appearance of Interleukin 1 $\alpha$  (IL-1 $\alpha$ ) Can Be Used To Assess Cytotoxic Damage In Cultured Human Keratinocytes Exposed To Bis-( $\beta$ -chloroethyl)-sulfide (BCES). Y. PU, P. Lin, F.L. Vaughan, and I.A. Bernstein.
- T-1007 Assay of Cytotoxicity of Antimicrobial Agents by Correlation of Keratinocyte Numbers with Optical Density of Crystal Violet. S.T. BOYCE, C.Q. Sheeler, and I.A. Holder.
- T-1008 Coordinate Regulation of Growth Factor Expression in Human Keratinocytes by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. K.W. Gaido, L.S. Leonard, and S.C. MANESS.
- T-1009 Delayed Expression of Cytotoxicity in Normal Human Epidermal Keratinocytes Treated With Shampoos. C. Juneja and C.W. Stott.

**CARCINOGENESIS**

- T-1010 Effect of cAMP and Magnesium on Four Biochemical Markers of Carcinogenesis. S. SHARMA, S. Zhu, and V.E. Steele.
- T-1011 In Vitro Chemosensitivity Assays of Fresh Human Tumors: Culture in Liquid Versus Soft Agar Medium. A. Leyva, L.A. WETMORE, C. Zalles, and A. Freeman.
- T-1012 Primary RTE Foci Inhibition Assay: Effects of Vitamin A Derivatives As Potential Chemopreventive Agents. B.P. WILKINSON, S. Sharma, J. Arnold, and V.E. Steele.
- T-1013 Screening of Eighty-Seven Compounds for the Inhibitory Effect on Carcinogen-Induced Poly(ADP)ribose Polymerase Activity in Human Foreskin Fibroblasts. G. WYATT, S. Zhu, S. Sharma, and V.E. Steele.
- T-1014 Screening of Eighty-Five Compounds Using Inhibition of Benzo(a)pyrene-DNA Binding in BEAS-2B Cells as a Biochemical Marker for Carcinogenesis.
- T-1015 Evaluation of Involucrin Expression in Human Epithelial Cells for Possible Utility for Screening Cancer Chemopreventive Agents In Vitro. E. ELMORE, C. Sun, J.A. Buckmeier, and J.L. Redpath.

**LYMPHOID SYSTEMS**

- T-1016 *In Vitro* Expression of Heat Shock Proteins Associated with Hyperthermia and Ionizing Radiation Exposure in Human B-Cells. S.L. SCHNEIDER, S.A. Fuqua, M. Szekeresova, and M.L. Meltz.
- T-1017 Development of Human B-Lymphocyte Cultures Secreting Antibodies to *Botulinum* Neurotoxin. C. WEBB, J. Testa, J. Middlebrook, and B. Butman.
- T-1018 Differential Mytomycin C-Induced Cell Cycle Inhibition and Apoptosis in a B and T Lymphoblastoid In Vitro Systems. S. BLOOM, M. Potchinsky, and D. Muscarella.
- T-1019 In Vitro Effect of Organochlorine and Organophosphate Pesticides on Human Peripheral Blood Mononuclear Cells (HPBMNC). V. RAMA KRISHNA and P.R. Rao.

**GENERAL TOPICS**

- T-1020 Quality Control of L929 Cells for Use in In Vitro Cytotoxicity Methods: A Test Battery to Reveal Passage-Dependent Alterations in Cellular Responses. J.F. HAMBERGER, C.J. Peters, and C.B. Jessee.
- T-1021 Induction of 70,000-Da Heat Shock Protein in HeLa Cells By Mercury. H. OSHIMA, T. Hatayama, and M. Nakamura.
- T-1022 Factors Influencing Metal Toxicity in Continuous Cell Lines. J.T. JONES, K.K. Divine, and M. Oshiro.
- T-1023 Interaction Between Ca, Cd, and Zn and the Actin Filaments in MDCK Cells. J.W. MILLS, G. Church, and J. LaCroix.
- T-1024 Induction of NOS Gene Expression by TPA in Primary Neonatal Rat Hepatocytes. U. ARMATO, M. Menegazzi, C. Guerriero, C. Cardinale, J. Wu, A. Carcereri de Prati, S. Mariotto, and H. Suzuki.
- T-1025 Immortalization of Human Endothelial Cells by SV40 Virus: Possible Cooperative Effect of Abnormal P53 Gene. D.R. UMBENHAUER, L. Toji, T.E. Johnson, R.B. Hill, and W.W. Nichols.
- T-1026 Cell Cycle-Dependent Modulation of Protein Kinase C (PKC)-Related Signal Transduction by 2,3,7,8-Tetrachloro-dibenzo-p-dioxin (TCDD). T.J. WEBER and K.S. Ramos.
- T-1027 In Vitro Cytotoxicity Testing: 72-Hour Studies with Cultured Lung Cells. F.A. BARILE and D. Alexander.
- T-1028 Comparisons of the Toxicity of 30 Chemicals as Measured by 68 Different In Vitro Toxicity Tests. C. CLEMEDSON, E. Abdulla, F.A. Barile, C. Chesné, R. Clothier, M. Cottin, R. Curren, P. Dierickx, M. Ferro, G. Fiskesjö, L. Garza-Ocanas, M.J. Gómez-Lechón, M. Gölde, B. Isomaa, A. Kahru, R.B. Kemp, G. Kerszman, U. Kristen, M. Kunimoto, S. Kärenlampi, K. Lavrijsen, L. Lewan, T. Ohno, G. Persoone, R. Petterson, R. Roguet, L. Romert, T. Sawyer, H. Seibert, R. Shrivastava, M. Sjöström, N. Tanaka, F. Zucco, and B. Ekwall.
- T-1029 H<sub>2</sub>O<sub>2</sub> Induced Perturbation of Neuronal Physiology and Morphology. J.M. Ryan, P.J.S. SMITH and M. Tytell.
- T-1030 Cellular Accumulation of Carotenoids by HepG2 and Caco-2 Human Cell Lines. K.R. MARTIN, M.L. Failla, and J.C. Smith, Jr.
- T-1031 Primary Rat Hepatocyte Cultures Aid in the Chemical Identification of Toxic Chaparral (*Larrea tridentata*) Fractions. D. PRITCHARD, W. Obermeyer, J. Bradlaw, W. Roth, T. Flynn, J. Yates, and S. Page.
- T-1032 In Vitro Assessment of Fumonisin B<sub>1</sub> Toxicity Using Reaggregate Cultures of Chick Embryo Neural Retina Cells (CERC). J. BRADLAW, D. Pritchard, T. Flynn, R. Eppley, and M. Stack.
- T-1033 In Vitro Cytotoxicity of Trypanocides. R. KAMINSKY, C. Schmid, and R. Brun.
- T-1034 Influence of Electromagnetic Fields (EMF) Upon the Transformation of *Trypanosoma cruzi* to the Infective Stage for Vertebrates. S.M. Krassner, B. Granger, and E. ELMORE.
- T-1035 Alternative Test Methods for the Detection of *Clostridium difficile* Toxin for the Budget-Minded Laboratory. K.C. KALMUS, J.F. Kenny, and G.W. Kalmus.

**SILENT**

- T-1036 In Vitro Cytotoxicity of Dichlone. H. BABICH, L. Blau, E. Borenfreund, and A. Stern.

- PS-1** Model Systems To Study Development And Malignancy: The Significance Of Cell-Extracellular Matrix Interactions. MINA J. BISSELL. Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA.

Throughout development, the differentiated phenotype is heavily influenced by dynamic and reciprocal interactions between the cells and the surrounding microenvironment. Over the last 15 years, we have begun to systematically elucidate the elements of this microenvironment in the mouse mammary gland. We have established that the extracellular matrix (ECM) in general, and the basement membrane in particular, play a significant role in regulating the expression of milk protein genes both in culture and *in vivo*. The following picture has emerged: a) the regulation is transcriptional; b) there are novel enhancers that respond to ECM; c) the enhancers are tissue-specific, but also function with heterologous promoters; d) the signals are transmitted through integrins, and they are responsive to changes in cell shape; e) the ECM component that is most important for such regulation is laminin; f) the laminin domain that is responsible has been identified; g) utilizing transgenic animals that express the activated form of the matrix-degrading metalloproteinase, stromelysin, we have shown that remodeling of the basement membrane is crucial both for branching morphogenesis and expression of milk proteins. We have also begun to apply to human cells the knowledge gained from the mouse model and we have succeeded in establishing a simple yet informative three-dimensional assay for distinguishing normal from malignant human breast cells. This distinction has been difficult to obtain in conventional cultures. We have applied this versatile technique to define the function of suppressor genes such as NM23. We are now in a position to define the stromal components of the breast and to search for meaningful markers in the context of a well-designed microenvironment and to elucidate the molecular mechanisms involved.

- PS-2** Differentiation And Tumor Suppression In *Drosophila melanogaster*. ELISABETH GATEFF. Institut für Genetik, Johannes Gutenberg Universität, 55099 Mainz, Germany.

Recessive mutations in about 30 developmental genes of *Drosophila melanogaster* are causally responsible for the malignant or benign tumorous growth of specific cell types. Affected by the various mutations are neuronal-, integumental-, blood cell-, and germ cell-precursors in the embryo, the larva, and the adult. The respective cells, devoid of the capacity to differentiate, continue to grow in an autonomous, invasive, and lethal fashion. Eighteen tumor suppressor genes are cloned. Some of them seem to be involved in signal transduction (*1[2]gl*, *Notch*, *fused*, *Hop<sup>Tum-1</sup>*), others in splicing and RNA-binding (*Sxl*, *b[2]gcn*). One tumor suppressor gene mediates the localization of specific mRNAs in the egg (*orb*). *Ovo* is possibly a transcription factor and *1(2)tid* belongs to the chaperonine machinery. *1(1) disc large-1* is found in septate junctions, and the *rpS6* gene encodes the ribosomal protein S6. Homologous mam-

malian counterparts have been identified for the following five tumor suppressor genes, *1(2)gl*, *dlg*, *rpS6*, *Hop<sup>Tum-1</sup>*, *Notch*. The one step causality between a gene mutation and the malignant or benign growth represents the unique advantage of the *Drosophila* tumor suppressor gene system.

- PS-3** The Role Of Homeobox Genes In Plant Development. CINDY LINCOLN, Sarah Hake, Bharat Char, Toshi Foster, Lauren Hubbard, David Jackson, Randy Kerstetter, Laurie Smith, Bruce Veit, and Erik Vollbrecht. USDA/ARS Plant Gene Expression Center, 800 Buchanan Street, Albany, CA 94710; and Department of Plant Biology, University of California, Berkeley, CA 94720.

Although plants and animals utilize fundamentally different developmental strategies, both systems require regulatory mechanisms that control the proper specification of cell fate. Homeobox genes in animals appear to act as control points during the processes of cell specification and pattern formation. The homeobox encodes a conserved DNA-binding homeodomain region; homeodomain proteins are believed to regulate the transcription of downstream target genes. Plant homeobox genes probably also participate in the regulation of important developmental decisions. The maize homeobox gene *Knotted-1 (Kn1)* is defined by a number of dominant neomorphic mutations that disrupt leaf development. In *Kn1* mutants and transgenic plants overexpressing *Kn1*, the fates of specific leaf cells are altered suggesting that this plant homeobox gene functions as a regulator of cell determination. *Knotted* related homeobox genes have been cloned from both maize and *Arabidopsis*. Many of these genes have been mapped to chromosomal locations and several show tight linkage to known mutations affecting various aspects of plant morphogenesis. The expression patterns of *Kn1* and several related genes have been examined in maize. Similar to animal development, the expression of *Kn1* and two related maize homeobox genes appears to predict or define morphogenetic events. For example, down regulation of *Kn1* in the shoot apical meristem occurs at the site of incipient leaf formation. By investigating the function of *Kn1*-like genes, we hope to determine what role homeobox genes play in plant pattern forming processes.

- P-4** No Abstract Submitted (J. McLachlin)

- I-1 Transformation Of Arthropods By Maternal Microinjection. M.A. HOY and J.K. Pesnail. Department of Entomology & Nematology, University of Florida, Gainesville, FL 32611.

Genetic manipulation of beneficial arthropods requires methods for efficient and stable transformation and knowledge of appropriate promoters and other regulatory elements required to obtain an effective expression of the inserted gene in both space and time. The number of genes that are cloned and of potential value for pest management programs is limited to single genes for resistance to pesticides or other toxins at present. Before transgenic arthropods can be developed with other complex traits, we must understand the underlying mechanisms and identify the critical genes involved in these processes. One factor hindering progress is the lack of a 'universal' transformation system that will provide a rapid and general system for introducing exogenous DNA into species for which little genetic information is available. Maternal microinjection, originally developed for a phytoseiid predator, appears to transform a parasitoid wasp at relatively high rates, and thus could be useful for transforming a variety of arthropods.

- I-2 The Use Of *Mariner* Transposable Elements As Insect Transformation Vectors. D.J. LAMPE. Department of Entomology, University of Illinois, Urbana, IL 61801.

The ability to easily and stably integrate foreign DNA into insect chromosomes requires a vector like a transposable element whose normal function is chromosomal insertion. To date, only the P transposable element of *Drosophila melanogaster* can be used for this purpose in insect cells and then only in *Drosophila* itself. We have discovered another short inverted repeat type transposable element, *mariner*, to be widespread in insect genomes. *Mariner* is approximately 1.3 kb in length and contains one open reading frame coding for a protein, presumably the *mariner* transposase, of about 345 amino acids. PCR analysis of over 600 insect species using primers corresponding to conserved amino acids flanking the middle third of the element suggest that 1) *mariner* is very widespread, occurring in every order examined (save the Odonata) and in other phyla, 2) at least 13 separate subfamilies of elements can be detected differing from each other by approximately 25-37% amino acid identity, 3) individual species can harbor more than one subfamily of *mariner* elements, and 4) phylogenetically distant species can contain elements that are virtually identical at the DNA level, suggesting recent horizontal transfer of the elements into their genomes. We have isolated full-length elements of one subfamily from the lacewing, *Chrysoperla plorabunda*, that appear to be intact and are developing this element as a transformation system first in *Drosophila melanogaster*, then hopefully in other insects. With its wide host range and seeming independence of host factors *mariner* may provide a general purpose transformation system for insect cell lines and germlines, ex-

tending the elegant molecular techniques developed in *Drosophila* to other insect species.

- I-3 Expression Of Foreign Genes In Tsetse Flies. S. AKSOY<sup>1</sup>, C.B. Beard<sup>2</sup>. <sup>1</sup>Yale University School of Medicine, Internal Medicine, New Haven, CT; <sup>2</sup>Division of Parasitic Diseases, Centers of Disease Control, Atlanta, GA.

The ability of insects to transmit disease causing pathogens can be modified if the insect genes involved in establishing susceptibility to infections can be identified and subsequently genetically modified. Alternatively, the introduction and expression of foreign anti-parasitic genes into insects can also interfere with disease transmission. Many arthropods including tsetse flies harbor symbiotic microorganisms permanently associated with their midgut cells. It is possible to exploit these natural bacterial symbionts as vehicles for the expression of foreign genes. We have been able to culture, characterize, and genetically transform these bacteria from tsetse. Since the symbionts are present in close proximity to parasites, anti-parasitic products can have an effect on midgut parasites. Desirable genes can be introduced and expressed with greater ease in these bacterial systems than in complex eukaryotic tissues. Symbionts can be manipulated *in vitro* and be reintroduced into insects and replace their native counterparts.

The presence of *Wolbachia*-like bacteria in the ovaries of some species suggests that cytoplasmic incompatibility phenomenon may be used to spread desirable anti-parasitic tsetse fly phenotypes in nature.

- I-4 Viral Expression Vectors For Mosquito Cells And Mosquitoes. J. CARLSON, K. Olson, S. Higgs, B. Afanasiev, and B. Beaty. Arthropod-borne and Infectious Disease Laboratory, Department of Microbiology, Colorado State University, Fort Collins, CO 80523.

The development of expression vectors from full-length clones of Sindbis virus (SIN) and the *Aedes densonucleosis* virus (AeDNV) allows packaging, delivery, and expression of novel genetic constructs in mosquito cells. In the SIN system, RNA is transcribed *in vitro* from plasmid DNA and transfected into BHK-21 cells to generate recombinant virus particles. These can subsequently be used to infect both mosquito cells and mosquitoes. In the AeDNV system plasmids with recombinant viral genomes are cotransfected into C6/36 (*Aedes albopictus*) cells with a plasmid containing the full-length AeDNV genome which provides replication and packaging functions. The use of these systems for the genetic manipulation of mosquito cells and mosquitoes will be discussed.

- I-5 Transgenic Mosquitoes: Contributions From Cell Culture. A.M. FALLON. Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108.

Application of transgenic technologies to insects has potential implications for control of both disease vectors and agricultural pests. Our work with the mosquito, *Aedes albopictus*, has focused on the development of cell culture approaches to facilitate the eventual expression of chimeric genes in transformed embryos. Given appropriate cloned genes and regulatory elements, recombinant constructs can be screened in cultured cells before the more time-consuming and labor-intensive embryo injections are performed.

Efficient transfection technologies for cultured mosquito cells are available, which makes use of polybrene or calcium phosphate to facilitate introduction of cloned DNA. Using these approaches, we have examined transient expression of the bacterial chloramphenicol acetyltransferase gene under the regulation of a temperature-inducible heat shock protein promoter from *Drosophila*. We have extended this work by developing chimeric plasmids containing the mosquito dihydrofolate reductase gene to recover stably-transfected clones. In the presence of methotrexate selection, stably transformed cells appear within 3 weeks; the number of transferred genes reaches 100-500 copies per cell.

Antisense expression of the mosquito dihydrofolate reductase gene decreases the survival and plating efficiency of transformed cells. By regulating antisense gene expression with a temperature-inducible promoter, we can recover transformed cells with novel phenotypes, whose properties may contribute to eventual transformation of insect embryos.

- I-6 Adhered Tissue From A Hexactinellid Sponge Reveals A Novel Transport System. S.P. LEYS. Department of Biology, University of Victoria, Victoria, B.C. V8W 2Y2.

Hexactinellids are deep water sponges that are unusual in possessing syncytial rather than cellular tissues. This difference becomes marked during the aggregation of dissociated tissue. Whereas cellular sponges aggregate by the active migration of individual cells, dissociated tissue from the hexactinellid *Rhabdocalyptus dawsoni* adheres to the substratum and extends broad, skirt-like lamellipodia and long filopodia which make contact with other tissue masses allowing the exchange of cytoplasm. In both kinds of sponges, so-called primary aggregates, small clusters of cells, detach from the substrate although aggregation may continue as primary aggregates come into contact. The detached aggregates, however, are too opaque to permit study of the cytoskeleton and associated organelles by light microscopy. In order to study the syncytial tissue of *Rhabdocalyptus* by light microscopy, we have developed a substrate of acellular tissue extract that causes the continued adhesion and spreading of the tissue for up to two weeks. Substrate adhesion is not species-specific; four species of demosponges and an-

other hexactinellid tested all adhere with differing ability to each other's extract. Adhered tissue from *Rhabdocalyptus* demonstrates vigorous cytoplasmic streaming along microtubule bundles within one hour and has formed a confluent syncytium within six hours of plating. The use of an acellular tissue extract to which sponge tissue readily adheres allows study of cellular functions in vitro. Most notably, use of this technique has revealed a motile system in hexactinellids that is unique among sponges.

- I-7 Recent Progress In Marine Sponge Cell Culture. S.A. POMPONI and R. Willoughby. Harbor Branch Oceanographic Institution, Division of Biomedical Marine Research, 5600 U.S. 1 North, Fort Pierce, FL 34946.

Development of methods for cell culture of bioactive marine sponges are in progress. Microbial contamination has been controlled through the use of physical separation methods and antibiotics. Sponge cells from several species have been successfully cryopreserved and remain viable after thawing. New techniques have been developed to monitor the physiological responses of non-cycling cells in culture using flow cytometry. A basal sponge cell culture medium has been developed for maintenance of primary cultures of several species of shallow water and deep water *Demospongiae* and *Calcarea*. To date, no continuous cell lines have been established; however, through the use of growth factors, transfer factors, hormones, lectins, organic nutrients, and trace elements, cultures of three species have undergone two to three population doublings within eight days. Scale-up of cultures from T-25 flasks to suspension culture in 100, 250, and 500 ml spinner flasks has been achieved. Current research efforts are focused on development of defined nutrient media and immortalization of cell lines.

- I-8 Novel Marine Alkaloids That Regulate Cell Growth And Differentiation. N.R. Shochet, D. Olchovsky\*, and I. SPECTOR. Department of Physiol. and Biophys., HSC, SUNY, Stony Brook, NY 11794; \*Department Med. A, Sheba Medical Center, Tel-Hashomer 52621, Israel.

We have previously found that six novel alkaloids purified from the Red Sea tunicate *Eudistoma sp.* are potent regulators of cell growth and differentiation and exert remarkable effects on cell shape (J. Cell. Physiol. 157:481-492, 1993). Chemically, the six alkaloids contain a fused tetracyclic pyrido [2,3,4-kl]acridine ring system that has been identified in other alkaloids isolated from diverse marine species. The functions of this novel family of secondary metabolites in the marine species themselves are unclear, nor is it clear whether they act via the same mechanism. Significant differences in the effects of the six alkaloids on cell shape that reflect differences in chemical structure indicate that more than one mechanism is involved. The effects of at least one of the six alkaloids appears to be mediated by the cAMP signaling system. This compound, debromoshermilamine (DBS), is the only one that mim-

icked the effects of cAMP analogs, and forskolin on growth hormone release from anterior pituitary cells which is a cAMP-mediated process. DBS also induced arborization in SV40 transformed SV-T2 cells, a singular morphologic response of many cell types to cAMP elevation. DBS did increase slightly total levels of cytosolic cAMP in cells, but did not stimulate cAMP synthesis like forskolin does. The results suggest that DBS exerts its growth regulatory effects by acting on one of the components of the cAMP signaling system, and that the other *Eudistoma* alkaloids exert their growth regulatory effects through different mechanisms, that presumably involve other signal transduction systems.

V-9 No Abstract Submitted (B. Rinkervich)

- P-1** Molecular And Cellular Aspects Of Meristem Organization In Vitro and In Vivo. IAN SUSSEX. Department of Plant Biology, University of California, Berkeley, CA 94720.

Root and shoot apical meristems are initiated in the plant embryo and function to produce the entire postembryonic organism by regulated processes of cell formation, morphogenesis, and differentiation. We are studying the regulation of meristem function in three ways: 1) characterization of genes that are expressed during meristem initiation, 2) examination of interactions between cells in different layers of the meristem, and 3) study of cell allocation from the meristem to the axis and lateral organs. We have identified and studied the temporal and spatial expression of patterns of about 60 genes that are unregulated during root meristem initiation in radish and *Arabidopsis*, and are identifying promoter regions that might be involved in the pattern of regulation. We have generated chimeric tomato plants to study cell layer interactions and have found that the innermost layer appears to regulate many features of meristem organization. We have constructed fate maps of the *Arabidopsis* shoot apical meristem to study cell allocation to derivative organs and have found that meristem cell fates can be altered in response to environmental signals.

- P-2** Expression Of The Maize Homeobox Gene *Knotted-1* In Transgenic Maize. R.E. WILLIAMS, Y. Lie, N. Sinha, S. Hake, P.G. Lemaux. University of California, Berkeley, USDA/Plant Gene Expression Center, Albany, CA 94710.

The *Knotted-1* locus is defined by several dominant gain of function mutations that alter leaf development. In wild-type maize plants, *Kn1* protein is present in nuclei of apical meristems and immature axes but is down-regulated as lateral determinate organs are initiated. The dominant *Kn1* mutant phenotype is characterized by outpocketings or knots which form on the lateral veins due to aberrant differentiation within the leaf blade. *Knotted-1*, under control of the 35S promoter, was introduced into tobacco. In the transgenic plants, overexpression of *Kn1* correlates with an alteration in leaf morphogenesis in a dosage-dependent manner (Sinha, Williams, Hake; *Gen. Dev.* 7:787-795, 1993). One drawback of studying gene expression in heterologous systems is the potential for the absence of important endogenous gene regulators, such as activators and suppressors, that may be present in homologous systems. We reintroduced the full-length *Kn1* cDNA into maize using a transformation system that allows the reproducible introduction of exogenous genes (Wan, Widholm, Lemaux; Proc. Annual Maize Genetics Conference, 1993, p. 5). Type I callus was bombarded with two constructs, one having a selective gene, the other with *Kn1* driven by the 35S promoter. The multiple independent transformed plants show a range of phenotypes which are similar to naturally occurring *Knotted-1* mutants and include localized knots on the surface of leaf blades and ligule displacement. The most severe

plants show an extreme reduction in overall plant dimension. At the Northern level, the amount of transcript directly correlates with the severity of the phenotype. The resulting plants are being examined by *in situ* hybridization to learn more about the regulation of *Knotted-1* and its role in plant development.

- P-3** Conifer Somatic Embryogenesis For Developmental And Cellular Studies. L.C. FOWKE, S.M. Attree, and P. Binarova. Department of Biology, University of Saskatchewan, Saskatoon, Canada S7N 0W0.

Suspension cultures of white spruce somatic embryos are being used to study 1) embryo development and 2) the cytoskeleton in mitotic cells. New maturation methods using abscisic acid and polyethylene glycol (PEG) yielded high quality somatic embryos resembling mature zygotic embryos. Electron microscopy and biochemical studies showed that mature somatic embryos had well-developed cotyledons and contained high levels of storage lipids and proteins. Embryos matured with PEG could be desiccated to moisture levels below that of zygotic embryos, stored and then imbibed and converted to plantlets at high frequencies. Large numbers of somatic embryos have been matured in a bioreactor. Embryogenic suspensions grow rapidly and provide excellent experimental material for studies of cell division. Conifers have highly focused spindles at prophase, similar to Bryophytes which are characterized by polar microtubule organizing centers (MTOCs). MPM-2, a monoclonal antibody which recognizes phosphorylated proteins of animal centrosomes was used as a probe for MTOCs in dividing cells of white spruce. MPM-2 staining was localized by both immunofluorescence and immunogold electron microscopy.

- P-4** *Bt1*, A Gene Critical For Normal Starch Accumulation In Vivo, Is Not Expressed In Cells Of Maize Endosperm Suspension Cultures. H. CAO<sup>1</sup>, T.D. Sullivan<sup>2</sup>, and J.C. Shannon<sup>1</sup>. <sup>1</sup>Plant Physiology Program, Penn State University, University Park, PA 16802; <sup>2</sup>Lab. Genetics, University of Wisconsin, Madison, WI 53706.

The maize *brittle-1* (*bt1*) locus on chromosome 5 was identified in 1926 as a mutation that decreased the amount of starch deposition (about 20% of normal) and increased sugars in the endosperm. We have isolated *Bt1* cDNA clones, determined the transit peptide for plastid targeting, measured the metabolite levels, made *Bt1* antibodies, and localized the *Bt1* protein in amyloplast membranes. The *Bt1* protein (a putative transporter or primer protein) is a cluster of integral polypeptides of about 40 kD and in normal kernels accounts for about 40% of the total amyloplast membrane protein. Maize endosperm suspension cultures have been used as model systems for studying starch biosynthesis. We used two maize endosperm suspension-cultured cell lines that were derived from inbred A636 and the *waxy* mutant. The starch content in A636 cell cultures was about 10% of dry weight and was increased some by adding more sucrose or CCC. We compared the *Bt1*

protein, *Bt1* transcript, and *Bt1* gene by western, northern, and Southern blotting in the two culture lines with those in normal and *waxy* kernels. The *Bt1* protein was detected in the microsomal membranes of developing kernels of A636 and *waxy* but not in the microsomal membranes of the two cell cultures, nor in amyloplast membranes isolated from highly purified amyoplasts isolated from A636 cell cultures. The *Bt1* protein was not induced in cultures supplemented with additional sucrose, CCC, or sucrose plus CCC. We were unable to detect *Bt1* mRNA in cells of the endosperm suspension cultures. Preliminary results of Southern blots indicate that the *Bt1* gene is present in the genome of the cultured cells. These results indicate that cells in the two endosperm cultures do not express the *Bt1*, a gene which controls starch accumulation *in vivo*.

**P-5** Phloem Differentiation In Plant Tissue Cultures. R.D. SJÖLUND. Department of Biological Sciences, University of Iowa, Iowa City, IA 52242.

The differentiation of phloem can be regulated in plant tissue cultures. Callus of *Arabidopsis* and *Streptanthus* (Brassicaceae) differentiate islands of phloem (and xylem) on media containing low levels (0.1 mg/L) of NAA or IAA in combination with either BA or kinetin (1.0 mg/L) and sucrose, glucose, or fructose. Phloem differentiation is inhibited if high (2-5 mg/L) levels of 2,4-D replace the NAA or IAA. The cellular development of sieve elements and companion cells in culture parallels that seen in the parent plants. Callus phloem does function in phloem loading, and high levels of solutes and hydrostatic pressures are developed *in vitro*. Callus sieve elements have been isolated as protoplasts and used to raise phloem-specific monoclonal antibodies that recognize phloem in intact plants. Immunoblots of proteins from cultures that differentiate phloem (phloem +) or not (phloem -) can be used to identify phloem-specific proteins. The ability to regulate phloem differentiation *in vitro* may facilitate the study of phloem-specific gene expression.

**P-6** Developmental Pathway(s) Of Xylem Tracheary Element Differentiation. G.F. PETER and I.M. Sussex. Department of Plant Biology, University of California, Berkeley, CA 94720.

In plants, a critical developmental transition occurs when cells leave the meristem, cease replicating and begin to differentiate into specific cell types. Because differentiation of many types occurs primarily in small regions near meristems and growing primordia, the study of cell differentiation *in vivo* is limited by the lack of early markers to identify differentiating cells. Thus, inducible *in vitro* differentiation systems have been developed to dissect the temporal sequence of cellular and molecular changes and to determine developmental characteristics and regulatory mechanisms. Many of these model systems rely on the ability of plant cells to access alternative genetic programs from already differentiated states. For example, up to 60% of the isolated *Zinnia elegans* leaf mesophyll cells can be induced to

become xylem tracheary elements within 72 h, without an intervening cell cycle. However, we know little about the mechanisms by which quiescent cells are reprogrammed nor about the developmental relationship(s) between normal differentiation from cells of meristematic origin and the apparent pluri/totipotency of differentiated plant cells. To investigate this relationship, we are isolating cDNAs expressed in a temporal series from transdifferentiating *Z. elegans* mesophyll cells, and determining whether these same genes are also expressed and in a similar temporal sequence in primary tracheary elements differentiating from cells of meristematic origin.

**P-7** Developmental Kinetics Of Carrot Somatic Embryo Culture. C.-M. CHI<sup>1</sup>, H. Vits<sup>2</sup>, E.J. Staba<sup>2</sup>, T.J. Cooke<sup>3</sup>, and W.-S. Hu<sup>1</sup>. <sup>1</sup>Department of Chemical Engineering and Materials Science; <sup>2</sup>Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455; <sup>3</sup>Department of Botany, University of Maryland, College Park, MD 20742.

Somatic embryo characterization is typically accomplished by assignment to a discrete set of developmental classes. This is performed either by direct, laborious, and subjective microscopic observation or by image processing. The quantitative description of developmental kinetics has been hindered by these limitations in data acquisition, the lack of adequate descriptors discriminating the relative contributions of size and morphology, and the population heterogeneity. Employing image analysis techniques, we have implemented a pattern recognition system capable of automatically assigning individual embryos to morphological classes and of constructing the property distributions of embryo populations. These property distributions are essential to describe the developmental population kinetics by statistical means. Comprehensive kinetics of somatic embryo population, in conjunction with the developmental kinetics of individual embryos, are key to further the understanding of population heterogeneity. Monitoring the development of embryo cultures with these techniques can lead to process improvement and to the formulation of quality control strategies for large-scale micropropagation.

**P-8** A Comparison Of External Morphology And Internal Anatomy Of Shoot Forming And Non-Shoot Forming Somatic Embryos Of Sweetpotato (*Ipomea batatas* [L.] Lam). K. PADMANABHAN, D.J. Cantliffe, R.C. Harrell, and D.B. McConnell. Horticultural Sciences Department, University of Florida, Gainesville, FL 32611.

Somatic embryogenesis of sweet potato produce mature embryos that develop into plantlets in basal nutrient medium. Lack of high regeneration efficiency of somatic embryos hinders its practical application in transgenics or synthetic seeding. Conversion experiments with mature embryos over a 20-day period revealed that 80-90% of the embryos formed roots while only 25-30% of them formed organized shoots, even

though their external morphology appeared conversion-competent. To address this issue, 425 mature embryos were harvested and subjected to computer-based image analysis before transfer to conversion medium. Out of this, 75 random samples consisting of embryos arrested at 1, 5, 10, 15, 20 days after culture were subjected to histological analyses according to standard procedures and stained with toluidine blue. Examinations of serial sections in comparison with zygotic embryo sections revealed that lack of organized shoot development could be attributed to the following abnormalities: 1) lack of an organized apical meristem, 2) a flattened apical meristem, 3) sparsity of dividing cells in the apical region, and 4) multiple meristemoids and/or diffuse meristematic activity throughout the embryo. Most of the embryos had normal vascular tissue and root development. This work will provide a means to distinguish shoot forming from non-shoot forming embryo morphologies for improved regeneration efficiency.

- P-9** Peanut Plant Regeneration Through *In Vitro* Culture Of Peg Tips And Ovules Of *Arachis hypogaea* And *A. duranensis*. Q.L. FENG, H.T. Stalker, and H.E. Pattee. Department of Crop Science and USDA-ARS, Department of Botany, North Carolina State University, Raleigh, NC 27695.

*In vitro* culture of embryos in *Arachis* is necessary to recover interspecific hybrids that abort soon after fertilization. *In Vitro* techniques have been developed to promote the development of embryos and to regenerate plants. Aerial peg tips (with the embryos, ovules, and peg meristems) of a cultivated species, *A. hypogaea* cv. NC 6, and a wild species, *A. duranensis*, were collected 7, 10, 14, and 21 d after self-pollination. They were cultured in the dark on combined MS and B5 media with various combinations of NAA, GA<sub>3</sub>, and 6-BAP for 12 wks. Ovules or seeds were then isolated from the developing pods and cultured on MS media with NAA and 6-BAP to recover plants. Results indicate that 10-day-old peg tips (with eight-celled proembryos) were optimal for achieving *in vitro* embryo development and pod formation in both species. Effects of the three growth regulators were variable for pod formation, embryo and ovule development, peg elongation, and callus and root production. High levels of NAA and 6-BAP induced calli and inhibited *in vitro* embryo development while GA<sub>3</sub> promoted slight peg elongation and facilitated pod formation. Moderate levels of NAA-induced root production and, in combination with very low levels of 6-BAP, induced pod formation and embryo development. Peanut fruits were obtained for both species from immature pegs *in vitro*. Several embryos had differentiated into cotyledons, embryonic axis, plumule, and radicle from the eight-celled stage. These ovules and seeds were germinated on MS medium and plants of *A. hypogaea* have been recovered. Relatively low rates of embryo development and pod formation were observed in *A. duranensis*, but seeds were also obtained from this species.

- P-10** Efficient Production Of Transgenic Barley Plants And Analysis Of Transgene Expression In Progeny. YUECHUN WAN and Peggy G. Lemaux. University of California-Berkeley/USDA-ARS, Plant Gene Expression Center and Department of Plant Biology, 800 Buchanan Street, Albany, CA 94710.

We have established an efficient and reproducible system to generate large numbers of independently transformed, self-fertile, transgenic barley (*Hordeum vulgare* L.) plants. Immature zygotic embryos, young callus and microspore-derived embryos were bombarded with either one plasmid containing *bar* and *uidA*, or in combination with another plasmid, containing a barley yellow dwarf virus coat protein (BYDVcp) gene. A total of 91 independent callus lines expressed functional phosphinothricin acetyltransferase, the product of *bar*. Integration of *bar* was confirmed by DNA hybridization in the 67 lines analyzed. Cotransformation frequencies of 84 and 85% were determined for the two linked genes (*bar* and *uidA*) and for two unlinked genes (*bar* and the BYDVcp gene), respectively. More than 500 green, fertile transgenic plants were regenerated from 36 transformed callus lines. T<sub>0</sub> plants in 25 lines (3 plants per line) were analyzed by DNA hybridization and all contained *bar*. Transmission of the genes to T<sub>1</sub> progeny was confirmed in the five families analyzed by DNA hybridization. Functional expression of *bar* was confirmed in some T<sub>1</sub> plants by resistance to herbicide treatment. A detailed analysis of transgene expression in progeny is under way.

- P-11** Production Of Fertile Transgenic Maize By Electroporation. T. MICHAEL SPENCER, Cheryl Montain Laursen<sup>1</sup>, Richard A. Krzyzek<sup>2</sup>, Paul C. Anderson, Thomas R. Adams, and Christopher E. Flick. Discovery Research, DEKALB Plant Genetics, 62 Maritime Drive, Mystic, CT 06355-1958; <sup>1</sup>6208 Hammersley Road, Madison, WI 53711; <sup>2</sup>R&D Systems, Inc., 614 McKinley Place, Minneapolis, MN 55413.

Fertile, transgenic maize plants were generated by electroporation of suspension culture cells that were treated with a pectin-degrading enzyme. Electroporation parameters were optimized based on transient expression assays. Cells from different maize suspension cultures, including a culture derived from a B73-related inbred, were electroporated with selectable marker genes resulting in high-frequency recovery of stably transformed callus lines. Plants were regenerated from numerous transformed callus lines and inheritance and expression of the introduced DNA in progeny was demonstrated.

- P-12** Production Of Fertile Transgenic Maize Plants By Silicon Carbide Fiber-Mediated Transformation. B.R. FRAME<sup>1</sup>, P.R. Drayton<sup>2</sup>, S.V. Bagnall<sup>1</sup>, C.J. Lewnau<sup>1</sup>, W.P. Bullock<sup>1</sup>, H.M. Wilson<sup>1</sup>, J.M. Dunwell<sup>2</sup>, J.A. Thompson<sup>2</sup>, and K. Wang<sup>1</sup>. <sup>1</sup>ICI Seeds, 2369 330th Street, Box 500, Slater, IA 50244; <sup>2</sup>ZENECA Seeds, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK.

A simple and inexpensive system for the generation of fertile, transgenic maize plants has been developed. Cells from embryogenic maize suspension cultures were transformed using silicon carbide fibers to deliver plasmid DNA carrying the bacterial *bar* and *gus* genes. Transformed calli were selected on media containing the herbicide bialaphos. Integration of the *bar* gene and activity of the enzyme phosphinothricin acetyltransferase (PAT) was confirmed in all bialaphos-resistant callus lines analyzed. Fertile transgenic maize plants were regenerated. The *bar* gene was transmitted to the progeny of bialaphos-resistant transformants in a Mendelian fashion. Localized application of herbicide to leaves of transgenic R0, R1, and R2 plants resulted in no necrosis, confirming the functional activity of the *bar* gene.

- P-13** Genetic Transformation And Gene Suppression In Seed And Vegetatively-Propagated Crops. NEAL COURTNEY-GUTTERSON. DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608.

The combination of genetic transformation and gene suppression technology is being used to generate novel fruit and vegetable varieties for commerce. The suppression method must result in phenotypes that are retained faithfully in seed or vegetable progeny. The transformation method must be able to produce fully transformed plants identical to the parental line. Sense suppression (Transwitch™) has been used already to create traits that are stable in model crops and in our near-term commercial products. This has been demonstrated over several generations and with large progeny numbers. Our current approach to achieving genetic transformation in less well-studied fruit crops, which relies on somatic embryogenic cultures, will be described.

- P-14** Approaches To Food And Feed Safety Assessments. STEPHEN R. PADGETTE, Roy L. Fuchs, Stephen G. Rogers, and Diane B. Re. Agricultural Group of Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198.

Several of Monsanto's genetically-improved crops have been field tested for multiple years and are in advanced stages of safety assessment. Our safety assessment framework focuses on several key areas: 1) characterization of the DNA/gene(s) inserted into the crop as well as the protein(s) produced as a result of the gene insertion; 2) determination of the levels of the newly expressed protein(s) in the appropriate tissues of the crop; 3) analysis of quality parameters of the feed/food

components relative to non-modified parental line; 4) processing studies for key feed/food uses, and analysis of the newly expressed protein(s) in the processing fraction(s); 5) animal feeding studies in rodents and other animals to assure the wholesomeness of the food/feed product; 6) mouse gavage studies with the newly expressed protein(s) to assess its safety; and 7) in vitro digestion studies to ensure that the newly expressed protein(s) are digested readily under stimulated mammalian digestive conditions. Utilizing this framework, advanced commercial crop candidates are evaluated for food and feed safety.

- P-15** Public Perception Of Biotechnology. T.J. HOBAN. Department of Sociology and Anthropology, North Carolina State University, Raleigh, NC 27695-8107.

Biotechnology has the potential to significantly enhance food production. However, this potential will be realized only if consumers accept the use of these new technologies as effective, safe, and ethical. Scientists and industry leaders need to better understand and address public perceptions of biotechnology. This project involved a national telephone survey conducted with 1,228 adults and a series of eight focus groups. In general, understanding of biotechnology is low, but interest is high. Most people appear positive about the general concept of biotechnology. However, certain applications in agriculture and food production may prove to be relatively unacceptable to consumers. This is especially true for animal applications and transgenic food products. Environmental, economic, and ethical issues will prove to be important for many consumers. Results suggest that consumer acceptance of some foods produced through biotechnology could be limited by insufficient knowledge, lack of confidence in government, moral objections to tampering with nature, and perceived environmental and food safety concerns. The future of biotechnology in food production and processing depends on a greater commitment to consumer education. Results of this project provide implications for public policies, future research, and product development.

- P-16** The Relative Safety Of Transgenic And Non-Transgenic Plants: How Do We Assess It? P.J. DALE. John Innes Centre, Colney, Norwich NR4 7UJ, United Kingdom.

Advances in molecular biology and transformation over the past decade have made it possible to modify plants in many novel ways. The essential difference between modifying plants by transformation compared with sexual hybridization, is that the choice of genes available through transformation is not limited by sexual incompatibility. Genes can be transformed into plants from a wide range of microorganisms, animals, and unrelated plant species. As a consequence it is possible to introduce into plants, types of genes that are outside the direct experience of conventional plant breeding. Because of this it is necessary to go through a process of risk assessment to determine the consequences of

the novel phenotype to human health and the environment. Various kinds of information are used for risk assessment and include a consideration of features of the crop, and characteristics of the transgene inserted. This procedure includes an assessment of the likelihood and consequences of cross pollination between transgenic crop plants and related species, and the distance over which pollen will travel. Experiments aimed at determining the degree of transgene movement from transgenic field plots will be discussed, along with the challenges of estimating the impact of a transgene in wild populations.

- P-17** Production Of Monoclonal Antibodies And Candidate Vaccines In Plants And Plant Cell Cultures. John Fitch. The Scripps Research Institute, 10666 N. Torrey Pines, La Jolla, CA.

Plants and plant cell cultures offer distinct advantages over alternative systems for production of proteins for therapeutic or immuno-protective uses. Two examples, monoclonal antibodies and hybrid molecules to be used to deliver antigenic epitopes, illustrate the efficient production of non-plant proteins in plant cells. Synthesis and assembly of monoclonal antibodies in transgenic plants has been demonstrated for antibodies recognizing a variety of antigens. When appropriate sequences are included in the introduced genes, the antibody is secreted to the extracellular space. Cell suspension cultures derived from such a transgenic plant accumulate assembled antibody in the culture medium up to microgram per milliliter quantities. This antibody is functionally equivalent by several criteria to hybridoma-produced antibody. However, minor structural differences between the plant cell- and hybridoma-derived antibodies have been shown. These differences may influence immunogenicity and/or biodistribution. Disease-related antigens have been expressed in plants for use as antigens in candidate vaccines. The antigenic regions have been expressed either in their natural context or as fusion proteins. Specific limitations of the whole plant expression systems may be overcome by production in cultured cells.

- P-18** Systems For Production Of Secondary Metabolites From Plants In Vitro. PAMELA J. WEATHERS. Department Biology/Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609.

Plants are a rich source of pharmaceuticals and other specialty chemicals, yet few products are currently under production. Many diverse systems exist for *in vitro* production including differentiated tissues (roots, shoots, etc.), immortalized cells, and cell suspensions. A variety of different bioreactors including stirred tank, rotating drum, perfusion, packed bed, trickle bed, and nutrient mist bioreactors are also available for cultivating this diversity of production crops. An overview of these production systems will be presented culminating in a case analysis of the projected economics of artemisinin production by *Artemisia annua* based on data from the literature and the author's laboratory.

- P-19** Natural Pigments And Medicinal Compounds From Anthocyanin-Rich Plant Cells. M.A.L. SMITH and D.L. Madhavi. University of Illinois, Urbana, IL 61801.

Anthocyanin-producing plants are a source of natural foods and food additives that are not only innocuous, but have health-promoting properties unrelated to nutritive value. Consumer concerns over synthetic food supplements and recent publicity lauding "designer foods" have stimulated research towards viable systems for producing these natural phytochemicals *in vitro*. Targeted cell cultures (*Vaccinium* and *Ajuga*) responded to chemical microenvironmental treatment with significant increases in pigment yield. Spectral irradiance treatments (physical microenvironmental) enhanced yield but did not alter the anthocyanin profile, although expression of key enzymes and other phenolic compounds in the pathway was modified. Explant source for callus induction (*in vitro* vs. *in vivo*; vegetative vs. reproductive) markedly regulated the expression of anthocyanins. Chemoprotective activity (both cardioprotective and anticarcinogenic) was observed in the total phytochemical extract from *Vaccinium*; a three-fold greater anticarcinogenic activity was found in the non-anthocyanin (phenolic) fractions. Measurement and control of the liquid culture bioprocess was approached using color machine vision (hue characterization) to quantify productivity (pigment concentrations and cell growth).

- P-20** Assay Of Alkaloids In Narcissus And Other Amaryllidaceae By HPTLC and HPLC. R.D. SHILLITO, I. Rouse, and N. Andersen. Ciba Biotechnology, 3054 Cornwallis Road, Research Triangle Park, NC 27709.

Narcissus and other Amaryllidaceae contain a number of alkaloids and other compounds. Interest in these alkaloids has recently increased due to their reported pharmaceutical and other useful properties (Martin, 1987, and refs. therein; Poulou, 1992). As part of a project on alkaloids, we were interested in assessing the spectrum of alkaloids available in commercially obtainable bulbs. Previous studies have used chemical methods (Wildman, 1968, and refs. therein.; Martin, 1987, and refs. therein), HPLC (Evidente et al., 1983), or immunological methods (Poulou, 1992) to determine alkaloid concentrations. However, these methods usually concentrated on determination of a single component. We developed quick and simple methods to extract and simultaneously determine alkaloids in 34 batches of bulbs of 29 different cultivars of Narcissus and related bulbs. The methods used are based on a simple extraction method and both Thin Layer Chromatography and HPLC to identify and quantitate Galanthamine, Dihydrogalanthamine (Lycoramine), Narwedine, Nivalidine, and Lycorine. These approaches can be used to a) rapidly quantitate alkaloids, or b) test batches of bulbs to determine if they are true to type.

**P-21** Large-Scale Production Of Secondary Metabolites From Plant Cell Cultures. P.G. KADKADE and E.J. Kane. Phyton Inc., 125 Langmuir Lab, 95 Brown Road, Ithaca, NY 14850.

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During the last decade, considerable attention has been drawn for utilizing plant cells as a source of economically important secondary metabolites. As a result, a variety of bioreactor processing systems have been developed for scaling-up plant cell cultures. These include growing the plant cells in an industrial scale fermentor in batch, semi-continuous, and continuous culture systems. The bioreactor systems, such as stirred tank, airlift or drum type fermentors have been used for the large-scale cultivation of plant cells and the production of secondary compounds. While investigations have demonstrated that plant cell culture processes are manageable at a large scale (up to 75,000 L), only limited successes have been achieved in directing cells to efficiently produce desired plant-derived compounds. There are yet many issues that need to be resolved when scaling up plant cell culture technology from bench level to commercial level production. These issues include the integration of several strategies used to stimulate and enhance production as well as the use of specific fermentor designs based on the type of cell culture necessary for production of the desired secondary metabolite. The plant genetic engineering approaches coupled with the ability to design processes that consistently result in high rates of product formation should allow the industry to exploit the full commercial potential of plant cell cultures.

**T-1** Drug Metabolism In Primary Cultures Of Adult Human Hepatocytes. Patrick Maurel, INSERM U-128, BP5051, 34033 Montpellier, France.

Among the numerous enzyme systems involved in the metabolism of xenobiotics, cytochromes P450 play a prominent role. These cytochromes are mainly expressed in the liver. They are able to oxidize an apparently unlimited number of compounds and, in some occasions, generate cytotoxic or genotoxic metabolites responsible for various pathologies including hepatitis and chemical carcinogenesis. The expression and function of these cytochromes might be affected by a number of factors, including physiological (hormones, growth factors, cytokines, etc.), pathological (infections, inflammation, hepatectomy, etc.), genetic (polymorphism of expression or function), and environmental (drugs, diet compounds, pollutants) factors. These various properties account for the wide interindividual variability exhibited by the human populations in response to drugs and environmental pollutants in terms of metabolism and toxicity.

In this communication, we will show how the use of human hepatocytes in primary culture allow to gather important information in the field of drug metabolism, side effects, and toxicity. Emphasis will be placed on the identification of the enzyme systems involved in the metabolism of several drugs, including cyclosporin A and proton pump inhibitors like lansoprazole and omeprazole, on the prediction of drug interactions as well as on the effects of these drugs on the expression of cytochrome P450 genes. In this context, emphasis will be placed on the comparison between our results obtained *in vitro* and the *in vivo* situation in man, as well as on the prediction, confirmation, and/or a *a posteriori* explanation of clinical observations on these drugs.

(Supported by Glaxo Ltd., Laboratories Houdé and Sandoz Ltd.)

**T-2** Metabolic Activation Of NNK By Human Cytochromes P450 Stably Expressed In Mammalian Cells. R. LANGENBACH, M. Cunningham, H. Tian, and C. Crespi<sup>1</sup>. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27707; <sup>1</sup>Gentest Corporation, Woburn, MA 01801.

We have studied the metabolism and nature of mutagenic events induced by the tobacco-specific nitrosamine, NNK [4-(methylnitrosoamino)-1-(3pyridyl)-1-butanone] in two mammalian cell lines stably expressing individual human cytochromes P450. The mammalian cell lines are the human lymphoblast line, AHH-1 and the Chinese hamster ovary cell line, AS52. The human cytochromes P450 expressed are CYP1A2, 2A6, 2B6, and 2E1. NNK can be metabolized by several pathways, including methyl and methylene hydroxylation, both of which lead to DNA reactive intermediates. The data indicate that the individual P450s are capable of producing varying amounts of the DNA methylating and pyridyloxobutylating intermediates. The mutational spectra of mutants obtained to date reflect the metabolic intermediates produced. These observations are dis-

cussed in relation to the organ-specific metabolism and carcinogenic effects of NNK.

**T-3** Sex- And Tissue-Specific Expression Of Highly Homologous Mouse Cytochrome P450s 2a-4 And 2a-5. Masahiko Negishi. Pharmacogenetics Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Cytochrome P450 represents a large family of structurally-related heme-thiolate oxygenases, enzymes that catalyze the oxidation of a large number of endogenous and exogenous chemicals. Since P450s play a key role in the metabolic detoxification and activation of environmental chemicals, their differential expressions and structural mutations alter defense of organs against toxicity and carcinogenicity of chemicals. Although mouse P450s 2a-4 and 2a-5 differ only by 11 amino acids within their 494 residues, they exhibit remarkably diverged expressions and activities. P450 2a-4 catalyzes steroid 15-alpha-hydroxylase activity, while P450 2a-5 catalyzes coumarin 7-hydroxylase activity. Furthermore, P450 2a-5 activates aflatoxin B1 more efficiently than P450 2a-4 does. These specificities are determined by the type of few critical residues, including those positions 117, 209, 364, and 481. P450 2a-4 is a female-specific enzyme in the liver, whereas it is male-specific in the kidney. P450 2a-5, on the other hand, is expressed in the livers of both sexes. Hepatotoxins such as pyrazole induce P450 2a-5 but not P450 2a-4. P450 2a-5 is overexpressed in hepatocellular carcinoma produced in 5,9-dimethyldibenzo[c,q] carbazol-treated females or TGF-alpha-transgenic males. In this presentation, I discuss the structural basis for understanding the function and regulatory specificities of these homologous P450s.

**T-4** Overview Of The Cellular Functions Of Stress Proteins And Their Potential For Use As Molecular Biomarkers. LAWRENCE E. HIGHTOWER. Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269-3044.

Molecular biomarkers have the potential to bridge the considerable gap between chemical analyses for the presence of toxicants and impairments of organismal physiology leading to effects on natural populations. Members of one set of stress proteins, known historically as heat shock proteins (hsp), are induced in response to chemical and physical stressors that cause cellular protein damage. As general indicators of proteotoxicity, hsp complement biomarkers of DNA damage or genotoxicity. Several points in the signal-response pathway of stress responses are amenable to measurement including transcription factor activation, induced RNA transcripts, induced proteins and their activities. In addition, the regulatory DNA sequences of the inducible genes can be attached to reporter proteins for ease of assay. Assays can be carried out on cultured cells and tissue samples from animals exposed in natural environments or in the laboratory. Cultured cells and test animals can be engineered with recombinant DNA con-

structs to produce transformed cells and transgenic animals carrying reporter genes under the control of the induction pathway used by the organisms normally to detect stressors. Current efforts are devoted to identifying the most promising components of various stress response systems for use as biomarkers and developing the most useful testing formats. The goals of this work include understanding the stability and regulation of various components and understanding the functions of the inducible proteins, since this basic information is crucial to proper interpretation of biomarker data.

- T-5 Biomonitoring Using An Organism's Own Cellular Stress Response System. J.A. RYAN and L.E. Hightower. Marine/Freshwater Biomedical Sciences Center, Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269.

All organisms, from bacteria and yeast to humans, respond to physical and chemical stressors by increasing the synthesis of a small group of "cellular stress proteins." We have developed a simple *in vitro* system for quickly screening environmentally relevant stressors to detect stress-induced proteins that are good candidates for biomarkers. Polyacrylamide gel electrophoresis was used to detect stressor-induced, concentration-dependent changes in cellular stress protein levels in two fish cell culture systems while simultaneous *in vitro* neutral red uptake cytotoxicity assays measured the stressor's effect on cellular physiology. There was a direct concentration-dependent relationship between sublethal cytotoxic effects and increases in stress protein levels. Increases of 50 to 200% were detected in two stress proteins (hsp70 and a 27 kD protein) from desert top-minnow, *Poeciliopsis lucida*, hepatoma-derived cell cultures as well as winter flounder, *Pleuronectes americanus*, kidney cell cultures as a result of short-term exposures to sublethal levels of cadmium or copper. These concentration dependent increases were not transient but persisted during the exposure period. When the hepatoma-derived cultures were exposed to cadmium and then allowed to recover, these stress proteins showed time-dependent decreases to near their original levels.

- T-6 Testing Of Xenobiotics And Environmental Samples Using Stress-Inducible Transgenic Strains Of The Nematode, *Caenorhabditis elegans*. E. PETER M. CANDIDO and Eve G. Stringham. Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver V6T 1Z3, Canada.

A stress-inducible promoter has been coupled to the *E. coli lacZ* reporter gene, and inserted into the genome of the free-living soil nematode, *C. elegans*. Strains carrying approximately 140 copies of the fusion gene per cell were derived and tested for their response to heat shock and a variety of xenobiotics. The activity of  $\beta$ -galactosidase can be assayed quickly and easily in these animals using either a) a histochemical stain that provides qualitative information on the cell and tissue types that are stressed, or b) a quantitative spectrophotomet-

ric assay that gives a read-out of the integrated stress response in the whole nematode population. Aqueous solutions are arsenite, and of cadmium, copper, lead, mercury, and zinc ions induced the stress response in these strains, as measured by  $\beta$ -galactosidase activity. Most of these agents give distinct tissue distributions of the induced enzyme activity. Organic inducers include the herbicide, paraquat, and the insecticides malathion, diazinon, and lindane. In addition to aqueous samples, soils can also be tested in this system, and we have begun screening soil and water samples from test sites at various locations in British Columbia. These results are being correlated with data derived from other biological tests on the same samples, such as Microtox and clam (*Macoma*) assays. (Supported by the B.C. Science Council and StressGen Biotechnologies Corp.)

- T-7 Use Of Transcriptional Activation Of Stress Response Genes To Establish "Molecular Fingerprints" Of Toxicants And Development Of A Reference Database. C.S. ORSER and S.B. Farr. Xenometrix Inc., 2860 Wilderness Place, Boulder, CO 80301.

Pro-Tox[E] and CAT-Tox[L], two stress gene assays composed of 16 bacterial and 15 mammalian genetic promoter elements respectively fused to reporter genes, have been developed at Xenometrix. The promoters in the assays respond to a wide variety of cellular stresses, including heavy metals, DNA alkylation, DNA scission, osmotic stress, polycyclic aromatic hydrocarbons, and protein perturbations through a colorimetric response that is sensitive, reproducible, and quantitative. Another stress gene assay that responds to DNA damage and damaging agents has been compiled in a separate assay, Pro-Tox[D], to specifically monitor genotoxic endpoints. The data collected from these and other stress response assays developed at Xenometrix are being compiled in a XenoMatrix Database, which promises to be a revolutionary means of assessing the relative toxicity of novel compounds and contaminants. Proprietary software is being developed to perform comparative toxicity assessment by multivariate comparisons.

- T-8 Cytochrome P450 Induction And Inhibition By Planar Halogenated Aromatic Hydrocarbons In A Fish Cell Line: Promise And Pitfalls For Environmental Testing. M.E. HAHN. Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Induction of cytochrome P4501A (CYP1A) is a well-known vertebrate response to planar halogenated aromatic hydrocarbon (HAH) exposure. Induction of CYP1A catalytic activity in mammalian cell lines has been used to determine relative potencies of pure compounds and "dioxin equivalents" present in complex environmental mixtures. Here we show the promise and potential pitfalls of such an approach, using data obtained with PLHC-1 fish hepatoma cells grown and assayed in 48-well plates. These cells express Ah receptors, which are known to control CYP1A induction in vertebrates. Dose-response relationships for induction of CYP1A catalytic

activity (ethoxyresorufin O-deethylase (EROD) by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and two planar chlorobiphenyls (CB-77, CB-126) were biphasic, with induction at low concentrations and an attenuated response at higher concentrations. The maximal level of EROD induction varied between compounds. In contrast, immunodetectable CYP1A protein increased monotonically with dose, and achieved similar maxima for the three compounds. Two mono-*ortho*-substituted chlorobiphenyls (CB-105, CB-118) were inactive as inducers of EROD activity or CYP1A protein. Possible mechanisms responsible for the loss of catalytic function at high doses of planar HAH include competitive inhibition and decreased heme synthesis. The results of these studies point out the utility of cell culture systems for determining taxon-specific structure-activity relationships and highlight the need for complementary measures of CYP1A induction. (Supported by grants from the Environmental Protection Agency and Sea Grant.)

**T-9** CYPIA1 Inductive Responses In Hepatoma Cell Lines As A Tool In Environmental Toxicology. D.E. TILLITT. National Fisheries Contaminant Research Center, U.S. National Biological Survey, 4200 New Haven Road, Columbia, MO 65201.

Clarification of the signal-response pathway and mechanistic aspects of dioxin-like toxicity has made *in vitro* models very useful tools in environmental toxicology. In particular, the inducible CYPIA1 response in cultured cells has provided a sensitive model system that allows fast, efficient, and reproducible information to be generated on individual compounds or mixtures. Our efforts have been to characterize CYPIA1 response in hepatoma cells for use in environmental studies.

We have developed a 96-well microplate format for cultured cells that allows use of the CYPIA1 inductive response in many aspects of toxicology. Transfection of reporter genes under the same regulatory control into cells has provided faster assays and allows us to probe species sensitivity differences. Potencies of PCBs, PCDDs, and PCDFs in a teleost hepatoma cell line are correlated with their potencies in whole organisms. Additionally, these investigations have demonstrated a quite different structure-activity relationship in teleosts as compared to mammals. Predictive models of the relative potency of PCDFs have been developed from physical and chemical descriptors and inductive capacity in the hepatoma cell lines. These partial least square models have been validated and subsequently used to predict the potencies of many untested congeners. This information can provide a relative ranking of toxicological importance and used to direct efforts for future testing. In field applications, the inductive response of the hepatoma cell cultures has provided a useful tool to integrate the effects of complex contaminant mixtures. These bioassays are complementary to chemical analyses and can help direct the analyses in some instances.

**T-10** Molecular Approaches To Retinoid-Induced Teratogenesis. J.F. Grippo, H.J. Kim, M.B. Zhang, and D.A. Lucas. Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ.

Retinoids exert their diverse physiological responses by interacting at the level of gene expression. Retinoid-bound nuclear receptors interact with DNA and cause changes in the expression of certain retinoid-responsive genes. Our laboratory has been interested in understanding the molecular mechanisms of retinoid-induced teratogenesis and we propose that retinoids induce teratogenesis by altering the expression of developmentally-important genes in embryos. We have developed an integrated approach to explore this hypothesis in which we first isolate and characterize retinoid-responsive genes from mouse embryo cDNA libraries. *In situ* hybridization methods are then used to characterize the effects of retinoid treatment on the expression of these isolated genes. Finally, the expression of the retinoid-responsive gene products are altered in the mouse embryo using transgenic model systems to determine the role those genes play in the teratogenic process. We have been working with a class of genes known as the homeobox genes that are retinoid-responsive, developmentally-important genes and have shown that one homeobox gene, Hox a-1, is strongly overexpressed in the mouse embryos following treatment of pregnant dams with retinoic acid. Of particular interest, overexpression of the Hox a-1 gene in transgenic animals leads to a reorganization of brain regions and we propose that retinoids that activate the expression of genes like that Hox a-1 gene in mouse embryos are likely to cause teratogenic malformations.

**T-11** Novel Molecular Approaches to Toxicology and Drug Discovery. S.B. FARR. Xenometrix Inc., 2860 Wilderness Place, Boulder, CO 80301.

Xenometrix, Inc., has developed a series of *in vitro* molecular assays employing both bacterial and mammalian cell systems, in which the expression of multiple genes are monitored during exposure of cells to novel compounds. An *E. coli* based assay (Pro-Tox [E]) monitors 16 different bacterial genes. A human liver cell-based assay (CAT-Tox [L]) monitors 14 different human genes, and a human DNA-damage responsive gene induction assay is currently in development. In addition to stress gene induction, Xenometrix is also monitoring genotoxicity in a yeast recombination assay (Yeast DEL) that is sensitive to a broad spectrum of DNA damaging agents, including those that are not detected in other short-term assays. *In vivo* assays include the development of a transgenic system that will permit the rapid assessment of DNA damage using modified shuttle vector technology. This Mutametrix assay permits the quantitative evaluation of DNA damage in transgenic rodents, nematodes and a selection of cell lines, employing the same basic assay format throughout.

These diverse molecular end points form the basis for detailed structure: function analyses of novel compounds. The assimilation of such "induction profiles" into the XenoMatrix database will enable compounds to be compared profile-by-profile, giving relative toxicity and efficacy information as part of a rapid screening program.

- T-12** Hepatic Peroxisome Proliferation: Mechanisms, Species Differences And *In Vitro/In Vivo* Correlations. B.G. LAKE. BIBRA Toxicology International, Carshalton, Surrey, SM5 4DS, UK.

A wide variety of chemicals (including therapeutic agents, herbicides, plasticizers, solvents, and natural products) have been shown to produce liver enlargement, hepatic peroxisome proliferation and induction of peroxisomal and microsomal fatty acid oxidizing enzyme activities in rodents. While peroxisome proliferators (PPs) are generally considered to be non-genotoxic agents, certain PPs have been shown to increase the incidence of liver tumors in rats and mice. Marked compound potency differences have been reported for both peroxisome proliferation and tumor formation. Recent studies have demonstrated that peroxisome proliferation in rodent hepatocytes is mediated through members of the nuclear hormone receptor superfamily. Many of the characteristics of PPs *in vivo* may also be demonstrated in *in vitro* systems such as primary hepatocyte cultures, liver slices, and certain cell lines. Such *in vitro* systems have been employed for mechanistic studies, structure-activity relationships, and for evaluation of species differences in response. Although peroxisome proliferation may be readily demonstrated *in vivo* and *in vitro* in rat and mouse hepatocytes, other species including the guinea pig, primates, and man are less responsive or even refractory to rodent PPs. (Supported by U.K. Ministry of Agriculture, Fisheries, and Food.)

- T-13** The AE Anion Exchanger Proteins: Structure, Function, Localization, And Regulation. S.L. ALPER, M.L. Chernova, Y. Zhang, B.D. Humphreys, A.S. Zolotarev, D. Yannoukakos, A. Stuart-Tilley, and L. Jiang. Molecular Medicine and Renal Units, Beth Israel Hospital; Department of Cell Biology, Harvard Medical School, Boston, MA 02215.

Chloride/bicarbonate exchange regulates intracellular pH, [Cl<sup>-</sup>] and volume in a wide variety of cell types. Much of this activity is thought to be mediated by proteins encoded by the AE band 3-related chloride/bicarbonate exchanger gene family. We have cloned AE1, AE2, and AE3 cDNAs, localized the encoded proteins of 900-1200 amino acids in length using anti-peptide antibodies, achieved heterologous functional expression of the cDNAs, and initiated a program of mutagenesis to study the mechanism and regulation of anion transport by the AE proteins. AE1 is primarily localized to erythrocytes and acid-secreting intercalated cells of kidney collecting duct. AE2 is widely expressed in epithelial cells, but highest levels have been found in choroid plexus, gastric parietal cells, and renal medulla. AE3 is expressed in

brain, heart, and other excitable tissue. Systemic changes in acid/base balance regulate the localization and accumulation of the AE proteins. The N-terminal cytoplasmic domains differ considerably in sequence, and have been proposed to serve as cell type-specific binding scaffolds or effectors for various cytoskeletal and, potentially, signalling elements. The C-terminal domains predicted to span the lipid bilayer 12-14 times each mediate Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange, and can do so without the cytoplasmic domains. We have expressed AE protein-mediated transport in *Xenopus* oocytes and in transiently transfected mammalian cells. Regulation of AE2 function in oocytes differs from AE1 in more sensitive regulation by intracellular pH, different regulation by extracellular pH, activation by hyperosmolarity, and regulation by guanine nucleotides and by the actin cytoskeleton. We are currently defining the domains of AE2, which mediate these distinguishing regulatory influences.

- T-14** Cell Culture Models Of Cystic Fibrosis Airway Epithelia. J.R. YANKASKAS. Division of Pulmonary Diseases, University of North Carolina, Chapel Hill, NC 27599-7020.

Cystic Fibrosis (CF) lung disease is caused by abnormal epithelial ion (particularly Cl<sup>-</sup> and Na<sup>+</sup>) transport, due to mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. Elucidation of these ion transport mechanisms and development of specific therapies for CF has been aided by primary and immortalized human airway epithelial cell cultures that retain the ion transport properties of the respective normal or CF cells *in vivo*.

Airway epithelial cells are isolated from excised nasal and bronchial tissues by enzyme disaggregation and cultured in serum-free, hormone-supplemented media. Cultures on plastic dishes proliferate, cultures in heterologous tracheal grafts differentiate morphologically, and cultures on permeable collagen matrix supports (CMS) develop ion transport properties similar to those of the native normal and CF epithelia. CMS cultures are the current "gold standard" *in vitro* ion transport model.

Immortalized cell lines were generated with SV40T and human papilloma virus (HPV) E6 and E7 genes, selected for increased growth potential, and screened for retention of polarized transport functions. Differentiated clones retain transport properties characteristic of normal and CF airways. HPV immortalized cells are quantitatively similar to primary cultures, whereas transport by SV40T cell lines is decreased. These cell culture models have been used to quantitate the driving forces for ion transport, localize CFTR and transport proteins, and to develop and test new therapeutic modalities.

(Supported by HL41983, DK46004, and the CF Foundation.)

- T-15** PTH-Stimulated Calcium Transport By Cultured Kidney Cells Requires Activation Of Protein Kinase A And Protein Kinase C. P.A. Friedman, F.A. Gesek, B.A. Coutermarsh, and S.M. Kennedy. Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755.

Parathyroid hormone (PTH) stimulates calcium absorption by renal distal convoluted tubules. The PTH receptor, which was recently cloned, is capable of coupling to adenylyl cyclase and to phospholipase C. However, it is not known if PTH actions require activation of both pathways. To identify the signaling mechanisms responsible for stimulating calcium entry in distal convoluted tubule cells, we applied a three-pronged strategy that involved determining the effects of PTH on formation of second messengers, inhibiting target enzymes with chemical or peptide blockers, and reconstituting the transport effect of PTH by addition of exogenous second messengers. Experiments were performed with primary and immortalized distal convoluted tubule cells. PTH[1-34] (10 nM) increased cAMP formation from  $44 \pm 6$  to  $178 \pm 19$  pmol/mg protein. PTH also increased diacylglycerol formation by 150% at 2 min and activated PKC by 50%. Addition of Rp-cAMPS, (0.3 mM) a specific blocker of PKA, or calphostin C (0.1  $\mu$ M), a blocker of PKC abolished PTH-stimulated  $^{45}\text{Ca}^{2+}$  uptake. Specific peptide inhibitors of PKA (PKI) and of PKC (PKC pseudosubstrate) were introduced into cells by transient permeabilization. PTH increased  $^{45}\text{Ca}^{2+}$  uptake equally in permeabilized and intact cells. In permeabilized cells, PKI and PKC pseudosubstrate abolished PTH-induced calcium uptake. Addition of forskolin (30 nM) or PMA (30 nM) alone had no effect on  $^{45}\text{Ca}^{2+}$  uptake. However, when added in combination, calcium uptake was stimulated to the same extent as with maximally effective concentrations of PTH. We conclude that stimulation of calcium uptake by distal convoluted tubule cells requires activation of both PKA and PKC. (Supported by NIH grant GM34399.)

- T-16** Entry Of Cholera Toxin (CT) Into The Polarized Human Intestinal Epithelial Cell Line, T84. W.I. LENCER. Pediatric GI, Children's Hospital, Harvard Medical School, Boston, MA 02115.

CT elicits a secretory response from intestinal epithelia by acting directly on epithelial cells and indirectly by action on submucosal nerves or other lamina propria cells. Toxin action within a single epithelial cell follows a complex series of events in which CT catalyses the ADP-ribosylation of the heterotrimeric GTPase Gs thereby activating adenylate cyclase, generating cAMP, and activating protein kinase A. The mechanism(s) of signal transduction, however, from apical receptor (GM1) to basolateral effector (adenylate-cyclase), or from lumen to lamina propria, remain incompletely understood. We have utilized the human intestinal cell line T84 as a model to study the mechanism of CT action on polarized epithelia. T84 cells grown as confluent monolayers on permeable supports display features of intestinal crypt cells and respond to CT with cAMP-dependent C1

secretion that can be detected electrically with a high degree of sensitivity and temporal resolution. This system is particularly relevant because the model requires that CT transduce a signal from the apical (physiologic) membrane and the response to CT in T84 cells reproduces the primary transport event of secretory diarrhea in humans: electrogenic C1 secretion. Utilizing this model system we have shown that signal transduction by apical CT involved endocytosis and vesicular transport of CT through sequential brefeldin A- and temperature-sensitive compartments (*J. Clin. Invest.* 92:2941-2951, 1993). We now examine whether toxin action requires vesicular transport of CT beyond these compartments to the basolateral membrane in a process termed transcytosis. Our data 1) show that apical CT enters the transcytotic pathway and CT B-subunit is delivered to the basolateral cell surface (suggesting transport to the lamina propria *in vivo*) and 2) suggest that transcytosis of CT may account for the transcellular signaling from the apical binding domain to the basolateral effector--adenylate cyclase.

- T-17** 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Stimulation Of Xenobiotic Toxicity In A Spontaneously Immortalized Line Of Human Epidermal Keratinocytes. R.H. RICE, A.A. Walsh, and L.A. deGraffenried. Department of Environmental Toxicology, University of California, Davis, CA 95616-8588.

Keratinocytes of the epidermis and other stratified squamous epithelia constitute a major barrier protecting us from the environment. A spontaneously immortalized keratinocyte line derived from human epidermis, which closely resembles normal epidermal cells in growth and differentiation, has been employed to study keratinocyte responses to certain model animal carcinogens. The mycotoxins aflatoxin B<sub>1</sub> and sterigmatocystin and the heterocyclic amine Trp-P-1 (a food mutagen) were highly toxic at  $\mu$ M concentrations. The potency of each agent was increased  $\geq 10$ -fold by treating the cells in the presence of 5 nM TCDD. Normal epidermal cells in parallel gave the same responses. The sensitization appeared attributable to enhanced cytochrome P450IA1 activity, induction of which was detected by northern blotting, since the cells did not display a toxic response to TCDD alone. We conclude SIK cultures are potentially useful models for testing the effects of agents whose toxicity is dependent upon metabolic activation by cytochrome P450IA1.

- T-18** Developing Better Defined Media For Toxicity Testing With Normal Human Cells. R.G. HAM. M.C.D. Biology, University of Colorado, Boulder, CO 80309.

Use of normal human cells for *in vitro* toxicity testing is currently limited by available culture media. Many new growth factors have been identified and recombinant DNA technology has made both new and older factors easier to obtain. However, development of defined media for normal human cells continues to lag. Medium development is a complex process that requires specialized knowledge of cellular growth requirements and

should ideally be undertaken in a laboratory dedicated to that purpose. Most granting agencies no longer consider medium development to be a good use of scarce research funds unless it is directly coupled to a "more important" project. Thus, most of the short-term responsibility for improving media used for toxicity testing will fall on those who are working in that area, and on commercial suppliers of media and cells used for toxicity testing. Major problems that still remain include the following: 1) Development of optimized nutrient media that will minimize requirements for macromolecular supplements for growth of a wider range of normal human cell types that could potentially be used for *in vitro* toxicity testing; 2) Replacement of serum, bovine pituitary extract (BPE) and other undefined supplements with known hormones, growth factors, etc. for a wider range of cell types; 3) Identification of factors that replace the remaining beneficial effects of serum and BPE in current defined medium systems; 4) Identification of any new growth factors that may be needed to replace residual requirements for undefined supplements; and 5) Development of improved ways to provide adequate lipid supplementation to normal cells grown in defined media with low protein content.

**T-19** Toward An *In Vitro* System For Testing Xenobiotic Effects On Mammary Function. M.C. NEVILLE. University of Colorado School of Medicine, Denver, CO 80262.

Milk secretion is a complex process involving coordination of diverse signals that promote rapid transcription of genes for milk protein and their translation into milk components that must be packaged and secreted apically from a tight epithelium. There is currently no *in vitro* system that reproduces this complex situation in its entirety. However, "normal" cell lines or of primary cells taken at the appropriate reproductive stage in the appropriate hormonal milieu and substratum provide cell cultures that are useful for a variety of *in vitro* tests.

Available systems include explants of mouse mammary gland primary cultures of cells from mouse or rat mammary gland, and certain cells lines, all from the mouse, that have retained characteristics of the differentiated phenotype. Each of these systems has advantages for the study of particular questions. For example, cultures of virgin rat mammary gland in reconstituted basement membrane demonstrate branching morphogenesis and functional differentiation (Hahm, H.A. and Ip, M.M. *In Vitro Cell. Dev. Biol.* 26:791-802, 1990) and should be useful for studies of differentiation. For studies of secretion itself, as well as of the integrity of cell junctions, we have found that a derivative of the Comma 1D cell line, called CIT<sub>3</sub> cells, offers many advantages when grown on membrane filters. The accumulation of secretory products in the apical medium is easily measured as is transepithelial resistance. Both apical and basal surfaces are accessible to rapid manipulation of medium components. CIT<sub>3</sub> cells have been found to actively transport the drug, nitrofurantoin, from basal to apical medium. However, there is a need for further

development of *in vitro* systems that more faithfully mimic the differentiated function of the mammary epithelium. (Supported by NIH grant HD 19547.)

**T-20** Immortalization Of Rabbit Kidney Proximal Tubule Cells In Serum-Free Medium. M. TAUB, C. Allen, H.J. Han, and J.H. Park. Biochemistry Department, State University N.Y., Buffalo, NY 14214.

Nephrotoxicity is a major problem in drug design and usage. Previously, we have examined the use of a primary rabbit kidney proximal tubule cell culture system for *in vitro* toxicology studies. More recently, we have been concerned with the use of an immortalized rabbit kidney proximal tubule cell culture system as an alternative to whole animal studies. Primary rabbit kidney cells were transformed with the pRSVT plasmid containing the SV40 early region genes. Clonal populations were selected for their ability to survive multiple passages in serum-free medium supplemented with insulin, transferrin, EGF, bovine pituitary extract, heparin, and BSA. Unlike untransformed populations that survived two passages, pRSVT transformed populations have survived more than 23 passages. Southern analysis indicated that the cells contain SV40 early region genes. Immortalized clones retained gamma glutamyl transpeptidase (GGT) activity. Na<sup>+</sup> dependent sugar uptake is retained albeit at a lower rate than in primary cells. Both GGT activity and Na<sup>+</sup> dependent sugar uptake are distinctive markers of the renal proximal tubule. Towards examining the potential use of these cells for *in vitro* toxicology studies, the effects of various nephrotoxins on cell viability. Similar to primary cultures, the LC<sub>50</sub> value for Ch<sub>3</sub>HgCl and HgCl<sub>2</sub> were 5 μM and 40 μM, respectively. The ranking of three different cephalosporin antibiotics with regards to toxicity was cephaloridine being more potent than cefazolin, followed by cephalothin.

**T-21** cAMP-Mediated Inhibition Of Phenobarbital-Inducible CYP2B1, CYP2B2, And CYP3A1 Gene Expression In Primary Rat Hepatocyte Cultures. J.S. SIDHU and C.J. Omiecinski. Department of Environmental Health, SC-34, University of Washington, Seattle, WA 98195.

We are currently using an optimized primary rat hepatocyte culture system to study phenobarbital (PB) inducible cytochrome P450 genes (CYP) *in vitro*. The mechanism of PB-mediated gene regulation is poorly understood, although it has been shown to involve transcriptional activation. Recently, the role of second messenger systems in regulating the expression of certain CYP genes has been demonstrated. Thus, the present study was undertaken to examine the role of second messengers in regulating PB-inducible CYP genes in primary rat hepatocyte cultures. Prior to PB-induction, cells were treated, either for a 1 h pulse or continuously with PB for 24 h, with increasing concentrations (0.1 μM to 100 μM) of various analogues and activators of intracellular cyclic adenosine monophosphate (cAMP). Following PB treatment for 24 h, hepato-

cyte RNA was isolated and probed with CYP2B1-, 2B2- and 3A1-specific oligonucleotides. Forskolin, glucagon, and the cAMP analogues, 8-bromo-cAMP, 8-chlorophenylthio-cAMP, and dibutyl-cAMP dramatically inhibited the PB-mediated induction of CYP2B1 and 2B2 in a dose-dependent manner. In contrast, only the cAMP analogues effected a similar inhibition of CYP3A1 mRNA levels. Forskolin, alone and in combination with PB, dramatically induced levels of CYP3A1 mRNA. As expected, elevated intracellular cAMP levels resulted in a dose-dependent induction of tyrosine aminotransferase (TAT) gene expression. The results of the present study and consideration of further signal transduction pathways may help elucidate mechanism(s) by which PB and PB-like agonists regulate specific CYP genes. (Supported by NIH grant GM32281.)

**T-22** Mechanisms Of Activation Of Neutrophils And Neutrophil-Mediated Toxicity. P.E. GANEY. Departments of Medicine and of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Activation of neutrophils (PMNs) by xenobiotic agents may be an important component of certain toxic responses. Stimulation of PMNs results in release of reactive oxygen species and other cytotoxic mediators that may contribute to tissue injury. A number of signal transduction pathways are important in the activation of PMNs, and these pathways may be the site of action of toxicants that alter PMN function. For example, exposure of rat PMNs to polychlorinated biphenyls (PCBs) *in vitro* stimulates both generation of reactive oxygen species and degranulation. Activation of PMNs by PCBs is associated with hydrolysis of phosphoinositides and is dependent on the presence of extracellular calcium. Thus, the mechanism of PCB-mediated activation of PMNs involves altered signal transduction. To study mechanisms of injury induced by activated PMNs, a coculture system was developed using hepatic parenchymal cells as a target for toxicity. Activation of PMNs in coculture results in cytotoxicity to the hepatic parenchymal cells. The mechanism of cytotoxicity is dependent on the specific PMN activator. The roles of proteolytic enzymes and of reactive oxygen and nitrogen species in the mechanism(s) of PMN-mediated cytotoxicity in this system are reported (ES05722 and ES04911).

**T-23** The Effects Of Toxicant-Induced Changes In Thiol Status On Transmembrane Signal Transduction In Human And Mouse T-lymphocyte Subsets. T.J. KAVANAGH, R.A. Ponce, A.J. Potter, D.L. Eaton, P.S. Rabinovitch, and A. Grossmann. Departments of Medicine, Environmental Health, Pathology and Comparative Medicine, University of Washington, Seattle, WA 98195.

We have previously published on the effects of glutathione depletion by several agents on transmembrane signaling and proliferation in human T lymphocytes. We have extended these studies to a comparison of the effects of toxic compounds from diverse chemical classes on transmembrane signaling in both mouse and

human lymphocytes. Human and mouse lymphocytes were isolated by density centrifugation, immunophenotypically labeled with fluorescent antibodies, and assessed for anti-CD3 (TCR)-mAb-stimulated calcium mobilization, or glutathione content by flow cytometry after treatment with various thiol reactive agents. Regardless of the chemical class, suppression of mitogen stimulated transmembrane signaling was highly correlated with the ability of these agents to deplete glutathione. These effects are seen in both mouse and human CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets. Mouse lymphocytes have less glutathione than human lymphocytes and are in general more sensitive to the suppressive effects of these agents on transmembrane signaling. These data indicate that glutathione status is an important determinant of T-cell response to antigen stimulation, and that exposure to thiol reactive agents may result in immunosuppression through interference with transmembrane signal transduction. (This work was supported by NIH Grants ES04696, AG01751, and ES07032.)

**T-24** Expression Of Class Alpha, Mu And Pi Glutathione S-transferases And Cytochromes P4501A1, 1A2, 2B1, 2B2, And 2E1 In Rat And Rabbit Primary Cultured Hepatocytes. R.F. NOVAK, R. Dwivedi, R. Zangar, and A. Gruebele. Institute of Chemical Toxicology, Wayne State University, Detroit, MI 48201.

Expression of the class alpha (Ya, Yc; 1,2 subunits, respectively), class mu (subunits 3, 4) and class pi (subunit 7) GSTs was monitored in primary rat hepatocytes maintained in culture on Vitrogen substrata in Chee's media for 3 to 8 days. GST levels and activity declined by ~75% during the first 48 h of culture. Class alpha GST expression was enhanced 1.5- to 2.0-fold in response to barbiturate, polycyclic aromatic hydrocarbon (PAH), antioxidant and oxidant inducers. Class mu GST expression was only marginally (~1.2- to 1.3-fold) increased. Class pi GST expression was below the level of detection until 72 h post-plating of cells, at which time a dramatic increase in this form was observed. Treatment of cultured hepatocytes with the Ca<sup>2+</sup> ionophore A23187 (0.5 μM) also resulted in increased class alpha GST expression. Altered Ca<sup>2+</sup> homeostasis resulted in enhanced expression of c-fos, c-jun which form the AP-1 transcription factor complex responsible for transcriptional activation of genes with cognate AP-1 recognition sites and may be involved in GST gene expression. Inhibitors of kinase activity failed to diminish GST expression in response to t-butylhydroquinone. CYP1A1, 1A2 expression was increased in response to PAH treatment, whereas barbiturate treatment enhanced 2B1/2B2 expression in cultured hepatocytes. CYP2E1 expression was also substantially increased in response to inducers in these culture cells. Although primary cultured rabbit hepatocytes remained responsive to inducers of CYP1A and 2B subfamilies, GST expression was unaffected by these agents, suggesting that the molecular mechanisms governing GST expression in the rabbit differ from those of the rat. (Supported by NIH grants GM42620 and ES03656.)

- V-1** Retinoid-Regulated Expression Of Tissue Transglutaminase In Apoptotic Cells. Peter J.A. Davies<sup>1,2</sup>, Laszlo Nagy<sup>1</sup>, Vilmos Thomazy<sup>1</sup>, and Roshantha Chandraratna<sup>2</sup>. <sup>1</sup>Department of Pharmacology, The University of Texas Medical School, Houston, TX; <sup>2</sup>Allergan, Inc., Irvine, CA 92713.

The biological effects of retinoids are mediated by two families of retinoid receptors, the RARs that bind all-trans retinoic acid and the RXRs that bind 9-cis retinoic acid. We have investigated the role of both receptor families in the expression of tissue Tgase and the induction of apoptosis in cells and tissues. Increased expression of either RAR- $\beta$ , or RAR- $\gamma$  in Balb 3T3 cells results in a marked increase in the induction of the endogenous tissue Tgase gene. In human promyelocytic leukemia (HL-60) cells, ligands that activate endogenous RXRs (9-cis RAR, 3-Methyl-TTNPB) both induce tissue transglutaminase expression and apoptosis. Ligands that activate endogenous RARs induce cellular differentiation but they induce neither Tgase expression nor apoptosis.

In summary, our studies suggest that induction of tissue Tgase is a common feature of cells undergoing retinoid-induced apoptosis. We believe this induction is paralleled by ligand activated transcription of the tissue Tgase gene, an effect that, depending on the cellular context, can be mediated by either RARs or RXRs.

- V-2** Regulation Of Gene Expression By Retinoids During Squamous Cell Differentiation. A.M. JETTEN. Cell Biology Section, Laboratory of Pulmonary Pathobiology, NIEHS, NIH, Research Triangle Park, NC 27709.

Squamous differentiation is accompanied by the induction of many squamous cell-specific genes including transglutaminase type I, cornifins, CL20, cholesterol sulfotransferase, a preprorelaxin-like gene and specific keratins. The induction of many of these genes is regulated at the transcriptional level. Promoter regions of several genes have been isolated and sequenced. Putative response elements have been identified in the promoter region of the transglutaminase type I, cornifin and relaxin genes. Retinoids suppress the induction of squamous-specific gene expression at both the transcriptional and posttranscriptional level. This regulation occurs at low (nanomolar) concentrations of retinoic acid. In addition, a specific chemical structure of the retinoid is critical for these responses. Using retinoids that selectively activate specific retinoic acid receptors and specific antagonists it was shown that genes controlled by retinoic acid are regulated by different retinoid signaling pathways. Many of the effects of retinoids on the expression of squamous cell-specific genes may be mediated by the nuclear retinoid receptors, RARs and/or RXRs. RAR $\alpha$  and RAR $\gamma$  mRNA are found to be expressed in both epidermal and tracheobronchial epithelial cells; however, RAR $\beta$  can only be induced in tracheobronchial epithelial cells. It was concluded that the induction of RAR $\beta$  does not mediate the suppression of squamous cell-specific genes but may be a require-

ment for the induction of the mucociliary pathway of differentiation in tracheobronchial epithelial cells.

- V-3** Strategies Towards Studying Retinoid Signaling In Cells And Mouse Embryos. E. LINNEY, M. Colbert, A. Darrow, T.M. Underhill, D. Cash, Q. Liu, L. Kotch, B. Smith, G.A. Johnson, and A. LaMantia. Duke University Medical Center, Durham, NC 27710.

We have used transgenic indicator mice to localize active retinoic acid receptors in the developing mouse embryo. To address the question of whether this activity corresponds to localized sources of retinoids and/or receptors, we have produced indicator cell lines that register the presence of retinoid *in trans*. In combination with this, we have used whole mount *in situ* hybridization to localize gene products associated with retinoid signaling. To explore what genes might be regulated by the receptors, we have identified and cloned receptor binding sites from total genomic DNA and have isolated putative promoter sequences corresponding to these DNA binding sites to begin to identify additional genes that might be regulated by the receptors. To explore three-dimensional structure in mouse embryos, we have created computer archives of mouse embryonic development. We are trying to use these various technologies to determine the roles retinoids play in the developing embryo.

- V-4** RARs And RXRs Are Required For Prevention Of Activation-Induced T Cell Apoptosis By Retinoic Acid. J.D. ASHWELL<sup>1</sup>, Y. Yang<sup>1</sup>, and R. Heyman<sup>2</sup>. <sup>1</sup>Laboratory of Immune Cell Biology, National Cancer Institute, Bethesda, MD 20892; <sup>2</sup>Ligand Pharmaceuticals, San Diego, CA 92121.

Retinoic acid (RA) interacts with two receptors, RARs and RXRs, which are ligand-regulated transcription factors. Whereas both all-*trans* RA and its 9-*cis* isomer are ligands for RARs, RXRs only bind 9-*cis* RA with high affinity. RXRs can heterodimerize with RARs to form transcriptionally active complexes. We have demonstrated that RA prevents activation-induced apoptosis in T cell hybridomas and thymocytes. 9-*cis* RA is about 10-fold more potent than all-*trans* RA in this regard, suggesting that RXRs are involved. All-*trans* RA also inhibits *ex vivo* apoptosis of peripheral blood lymphocytes from some patients infected with HIV. To investigate the molecular mechanism of RA effects on activation-induced apoptosis, RXR $\beta$  or a dominant-negative form of RXR $\beta$  was transfected into T-hybridoma cells. Whereas overexpression of RXR $\beta$  made the cells more sensitive to the protective effects of 9-*cis* RA, expression of the dominant negative RXR $\beta$  made the cells resistant. Synthetic compounds that selectively bind one or the other receptor were also used to prevent apoptosis. The results indicate that an RXR/RAR heterodimer is responsible for the effect of RA on activation-induced apoptosis. Furthermore, it appears likely that both subunits of the dimer must be independently bound by ligand for a functional complex to form.

- V-5** Functional Activity Of Long-Term Normal And SV40 Transfected T Lymphocyte Cultures. D.M. Murasko and Q.C. Ryan. Department of Microbiology and Immunology, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Among the most consistent changes associated with increasing age is a decreased ability of T lymphocytes to proliferate in response to mitogenic or antigenic stimuli. Although this decreased proliferation is most commonly demonstrated in short-term cultures, we have shown that human T lymphocytes in culture undergo cellular senescence similar to that observed in fibroblasts. After primary stimulation with PHA and subsequent culture in IL-2, lymphocytes from young subjects (20-30 years old) undergo 25-40 population doublings (PD), while lymphocytes from aged individuals (65-90 years old) only demonstrated 10-24 PD. This senescence of T lymphocytes can be postponed by transfection with SV40 large T, resulting in PD of up to 170 for young and 65 for old lymphocytes. Transfected T cells of both young and elderly subjects appear to display normal T cell function; they cease doubling upon removal of IL-2; respond to mitogen stimulation and produce IL-2 and IFN- $\gamma$  in the presence of autologous adherent mononuclear cells during proliferation; and express both IL-2 and transferrin receptors similar to that observed in mitogen stimulated nontransfected T cells. More than 90% of transfected cells are CD4+ and express the naive T cell marker, CD45RA. Interestingly, we were unable to stably transfect CD8+ cells with SV40 large T. Therefore, we conclude the SV40 large T transfected CD4+ T lymphocytes maintain normal function and activated profile, while demonstrating an extended lifespan. (Supported by NIH AG03934.)

- V-6** Senescence Of Adrenocortical Cells In Culture. PETER J. HORNSBY. Huffington Center on Aging, Baylor College of Medicine, Houston, TX 77030.

Like human fibroblasts, human and bovine adrenocortical cells exhibit a finite proliferative potential in cell culture. Over a period of 30-40 population doublings for human cells and 100-120 population doublings for bovine cells, adrenal cells in culture exhibit several changes in differentiated function gene expression, accompanied by changes in DNA methylation of both unique copy genes and satellites. The relationship of methylation changes to differentiated gene expression is currently under investigation. Changes in gene expression can be divided into two phases: an early phase which results from the lack of appropriate factors in cell culture and a later phase of phenotypic switching in which cells change to a nonexpressing state with a certain probability. The molecular nature of phenotypic switching is unknown, although plasmids with regulatory sequences from genes that undergo switching still function well when transfected into late passage cells. The cessation of replication in adrenal cells strongly resembles that of fibroblasts. Both human and bovine adrenal cells express high levels of the senescence specific mRNA for SDI1. SDI1 expression is not detectable in the intact adrenal

cortex, but shortly after the cells are placed in culture, it becomes detectable and increases as the cells progress to senescence. In several experiments, markers of cell proliferation and of cellular senescence such as SDI1 have been shown to be dissociated from changes in differentiated function gene expression on a cell-by-cell basis. This has also been shown by the use of SV40 T antigen, which extends proliferative potential in adrenocortical cells as it does in fibroblasts.

In summary, replicative senescence in adrenocortical cells resembles the process in fibroblasts, but changes in differentiated gene expression occurring over the same time span in culture are separate events that are not secondary to the changes in replication.

- V-7** The Fibroblast Growth Factor (FGF)-1 Signaling Pathway Is Defective In The Interleukin (IL)-1 $\alpha$ -Mediated Pathway Of Human Umbilical Vein Endothelial Cell (HUVEC) Senescence. X. Hu, S. Garfinkel, and T. MACIAG. Department of Molecular Biology, Holland Laboratory, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855.

The FGF gene family is currently comprised of two signal sequence (sp)-less prototypes; FGF-1 (acidic) and FGF-2 (basic) and seven related members, the majority of which contain signal sequences to direct their secretion. FGF-1 is an angiogenic factor and the growth of HUVEC is dependent upon the presence of exogenous FGF-1. The function of FGF-1 as a HUVEC mitogen is antagonized by the sp-less IL-1 $\alpha$  and the generation of the quiescent HUVEC senescent phenotype correlates with the exaggerated intracellular activity of the IL-1 $\alpha$  precursor. Because extracellular IL-1 $\alpha$  functions to repress extracellular FGF-1 activity in young populations of HUVEC and high levels of intracellular IL-1 $\alpha$  may function to repress HUVEC growth in old HUVEC populations, we examined these populations for defects in the FGF-1 signal transduction pathway. Prior studies have shown that the ability of FGF-1 to induce an increase in the steady state mRNA level of the immediate-early gene, c-fos, is not altered in senescent HUVEC populations; evidence that the immediate-early component of the FGF receptor (R)-1 signaling pathway is not compromised by senescence. In addition, we have also shown that c-src and its major substrate, cortactin, a cytosolic protein containing a SH-3 domain and multiple helix-turn-helix domains are phosphorylated on tyrosine residues in response to FGF-1 during mid-to-late G<sub>1</sub>. Because c-src and cortactin are involved in the FGFR-1 signaling pathway, we examined the ability of young and senescent HUVEC populations to phosphorylate c-src and cortactin in response to FGF-1 late in G<sub>1</sub>. Using immunoprecipitation and immunoblot strategies, we observed that c-src and cortactin tyrosine phosphorylation are diminished in senescent but not in young HUVEC populations. We therefore suggest that the IL-1 $\alpha$  dependent senescent HUVEC phenotype involves a defect in FGF-1 signaling during the late G<sub>1</sub> phase of the HUVEC cycle. (Supported by NIH AG07450.)

**V-8** Cellular Senescence And Cell Cycle Regulators. M. NAKANISHI, S. Venable, and J.R. Smith. Department of Molecular Virology, Baylor College of Medicine, Houston, TX 77030.

SDI1 was cloned from a cDNA library of senescent human diploid fibroblast based on its ability to inhibit DNA synthesis in rapidly proliferating young fibroblasts. SDI1 has been found to inhibit the kinase activity of cyclin-Cdk complexes and to be activated by p53. SDI1 is upregulated in senescent cells about 20-fold compared to young proliferating cells. It is also increased by about 20-fold when young cycling cells are made quiescent by serum deprivation. Cells made quiescent either by serum deprivation or density inhibition can be made to transit the cell cycle by induction of antisense SDI1 from a stably integrated construct. SDI1 appears to inhibit the phosphorylation of Rb and thus the entry of cells into S phase. Down regulation of SDI1 is sufficient to drive cells through the cell cycle in the absence of other mitogens. The failure of senescent cells to sufficiently down regulate the level of SDI1 mRNA may be responsible for their inability to synthesize DNA.

**V-9** Automated Cell Culture Systems For The Space Shuttle. W.P. Wiesmann, L.A. Pranger, E.S. Delaplaine, and T.C. Cannon. Walter Reed Army Institute of Research, Washington, DC 20307.

Gravity may influence cell differentiation through changes in the extracellular matrix and integrin linked cytoskeletal signal transduction. To understand how physical forces affect cellular function, we have conducted cell culture experiments on the Space Shuttle using an automated cell culture system. The culture hardware contains multiple, parallel, hollow fiber cartridges, pumps and computer controls necessary to deliver nutrients, fixatives, CO<sub>2</sub>/O<sub>2</sub>, collect fractions and monitor system performance. We are currently modifying the instrument to provide greater capability to monitor cellular growth, viability, cell activation, differentiation and transformation in space through the addition of fiber optic sensors for pO<sub>2</sub>, pCO<sub>2</sub> and pH and a digital CCD high resolution microscope. The microscope electronics will either store digital images or downlink video signals that will transfer command and control of the microscope to scientists on the ground. Eventually, the microscope will have quantitative fluorescence capability. Studies with fluorescent dyes are planned to monitor the production, surface expression and secretion of peptides, proteins and gene products and measure rapid fluctuations in intracellular Ca<sup>++</sup> and membrane potential that may change in cells exposed to microgravity. These and other innovations in cell culture chambers and new biomaterials specifically made for space studies could improve the operation of large scale bioreactors, automate *in vitro* toxicological testing, and enhance tissue engineering and biosynthesis capabilities on the Earth.

**V-10** No Abstract Submitted (R.M.Wightman)

**V-11** Microphysiometry: Rapid Bioassays Based On Changes In Cell Metabolism. J. WALLACE PARCE. Molecular Devices Corp., 4700 Bohannon Drive, Menlo Park, CA 94025.

We have developed a technique (microphysiometry) to measure the rate of acid production, in real time, from a small volume (1 to 2 µL) of cells. Acid production is linked to the rate of energy consumption by the cells because the predominant acidic species produced are lactic acid and CO<sub>2</sub>. Using this technique, we discovered that measurable changes in the rate of acid production occur as a result of triggering practically all classes of functionally coupled cell surface receptors. Furthermore, measurement of the rate of acid production as a function of receptor ligand concentration produces dose response curves equivalent to those obtained using second messenger assays. Most recently, we have found that the kinetics of changes in the rate of acid production reflect the kinetics of changes of levels of second messengers in the cell. These data suggest that it is possible to perform receptor pharmacology studies rapidly, using relatively small numbers of cells.

**V-12** ECIS: An Electrical Method To Continuously Monitor Morphology And Motion Of Cells In Culture. C.R. KEESE and I. Giaever. Rensselaer Polytechnic Institute and Applied BioPhysics, Inc., Troy, NY 12180.

Great advances have been made in quantifying biochemical and physiological activities in cultured cells. It has, however, been difficult to quantify changes of cell morphology. A method has now been developed that can continuously and non-invasively track morphological changes of adherent cells and provide quantitative data from both sparse and confluent cultures. In Electric Cell-substrate Impedance Sensing (ECIS), cells are cultured on small (0.001 cm<sup>2</sup>) gold film electrodes whose impedance is measured with a 1 micro amp current, generally at 4000 Hz; normal tissue culture medium serves as the electrolyte. When cells attach and spread on these electrodes, their insulating membranes constrain the current and force it to flow beneath and between the cells. This results in large impedance changes. Furthermore, alterations in cell morphology give rise to variations in impedance that can be numerically analyzed to report levels of cell motility and, indirectly, cell metabolism. The approach is exceedingly sensitive and is capable of detecting changes in cell morphology on the order of nanometers, well below the resolution of an optical microscope. In addition to use as a general research tool to study cell spreading and motility, several potential applications of ECIS will be discussed. These include *in vitro* toxicology, drug efficacy and discovery, mammalian cell reactor monitoring, and determination of metastatic potential.

(Giaever, I. and Keese, C.R. ; Nature 366 591-592; 1993.)

- V-13** Multicellular Reorganization By Single Cells On Extracellular Matrix. M.H. BARCELLOS-HOFF. Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

Primary culture of mammary epithelial cells on a basement membrane matrix indicate that multicellular architecture has a role in regulating gene expression; furthermore, some functions of epithelial cells, such as compartmentalization, can only be established in three dimensions. The reconstitution of a luminal compartment by dissociated mammary epithelial cells provides an excellent opportunity to study histotypic reorganization in three dimensions. Mammary epithelial sphere formation on reconstituted basement membrane matrix in serum-free media was analyzed using phase contrast time-lapse videomicroscopy. Following attachment, four stages of multicellular reorganization are observed during the first 24 h: 1) cell elongation, 2) formation of networks of chained cells, 3) contraction of networks, and 4) compaction of resulting aggregates into spheres. This rapid reorganization is amenable to temporal and functional dissection of the molecular mechanisms by which reorganization is guided. For example, certain events during mammary epithelial cell reorganization on extracellular matrix are mediated by cell surface  $\beta$ -1,4-galactosyltransferase, a receptor that binds N-acetylglucosamine residues on glycosylated proteins. Cell elongation and network elaboration depend on cell surface galactosyltransferase while attachment, network contraction and compaction are independent. Further dissection of the sequence and interdependence of molecular mechanisms used during reorganization in culture may shed light on epithelial morphogenesis *in vivo*.

- V-14** Stromal Interactions In Growth And Adhesion Of Breast Carcinoma Cells: Role Of Growth Factors And Extracellular Matrix. B.E. ELLIOTT<sup>1</sup>, B. Bhardwaj<sup>1</sup>, R. Lall<sup>1</sup>, D. Leopold<sup>1</sup>, N. Rahimi<sup>1</sup>, R. Saulnier<sup>1</sup>, M. Park<sup>2</sup>, and T. Nakamura<sup>3</sup>. <sup>1</sup>Cancer Research Laboratory, Queen's University, Kingston, ON, Canada K7L 3N6; <sup>2</sup>Molecular Oncology Group, McGill University, Montreal, PQ, Canada; <sup>3</sup>Osaka University School of Medicine, Osaka, Japan.

We have examined the role of adipocytes, a dominant breast stromal cell type, in growth of a murine mammary carcinoma, SP1. 3T3-L1 adipocytes, but not preadipocytes, stimulated colony growth of SP1 cells in 0.36% agar. Both adhesive and mitogenic components were required for colony growth. In SP1 colonies, fibronectin (FN) appeared to be deposited extracellularly in the form of microfibrils; no fibrils were observed with SP1 cells grown in monolayers. Addition of 1-10  $\mu$ g/ml of the 70 kd amino-terminal fragment of FN, which blocks FN fibril formation, inhibited SP1 colony formation. Immunoprecipitation of <sup>125</sup>I surface-labeled cells demonstrated that the most abundant integrin types expressed on SP1 cells are the  $\alpha_5\beta_1$  and  $\alpha_v\beta_1$  FN receptors. The mitogenic activity of 3T3-L1 adipocytes was found to be mediated by hepatocyte growth factor (HGF). Antibody against HGF (15  $\mu$ g/ml) completely abrogated stimula-

tory activity in conditioned medium (CM) from 3T3-L1 adipocytes. Western blotting revealed an abundance of HGF in 3T3-L1 adipocyte CM, and of HGF receptor (*c-met*) in SP1 cells. Together, these findings indicate that FN fibril deposition in the tumor and secretion of HGF by adipocytes may be important regulatory events in the paracrine stimulation by the stroma of growth of breast carcinoma cells. (Supported by NCI(C) and MRC.)

- V-15** Progress In Studying The Growth And Cycling Of Hair Follicles. A.E. BUHL, D.J. Waldon, T.T. Kawabe, and K.E. Kappenman. Biochemistry, The Upjohn Company, Kalamazoo, MI 49007.

Hair follicles are an intriguing skin appendage that produces abundant, highly differentiated epithelium (hair) and also undergoes periodic remodeling (cycling). This organ provides a unique opportunity to study epithelial growth and differentiation, stem cells, and tissue reorganization. To investigate this unique biological system we compared follicular function in submerged and air-interface organ cultures with transplanted follicles *in vivo*. Submerged organ cultures of neonatal mouse vibrissa follicles with minoxidil treatment maintained function for 3-4 days; epithelial cells continued proliferation and differentiation with new hair-specific keratin. Without drug, differentiation stopped and epithelial cells began apoptosis. In contrast, vibrissa follicles cultured at the air-surface interface on Gelfoam® maintained function for 10 days without drug. Other substrates, including kidney capsule, were less effective indicating complex signaling from the surrounding dermis of the follicle. Follicles failed to cycle under any *in vitro* conditions but cycling occurred in explants to the kidney capsule. Recombination of follicular components under the kidney capsule also demonstrated the capacity for renewed organogenesis and suggested a specific location of stem cells. These results provide a firm basis for expanding efforts to develop organ conditions to study cycling.

- V-16** Liposome Targeting Of Functional DNA To The Hair Follicle Of Histocultured Skin: A Model For Gene Therapy Of Hair Growth. Lingna Li, Valeryi Lishko, and Robert M. Hoffman. AntiCancer Inc., 7917 Ostrow Street, San Diego, CA 92111.

Liposomes have been widely and successfully used as delivery systems to transport macromolecular substances into the cell which cannot normally cross the plasma membrane. We have recently reported that calcein-dye entrapped and melanin-entrapped liposomes specifically targeted hair follicles in histocultured intact skin. We report here utilizing DNA liposomes to target functional DNA to the hair follicle itself as a model of gene therapy of the hair growth processes. One kb DNA isolated from a mouse genomic DNA library labeled with <sup>35</sup>S was entrapped into phosphatidylcholine (PC) liposomes by freezing and thawing. Mouse skin, histocultured on collagen sponge gels was incubated with the liposomes for 44 hours. A solution of naked-[<sup>35</sup>S]DNA at the same concentration as was used in the liposome preparation served as the control and was also incubated with

the skin histocultures. The efficiency of liposome delivery of [<sup>35</sup>S]DNA to hair follicles was analyzed by autoradiography. The results showed that both the percent of [<sup>35</sup>S]DNA-labeled follicles per 20X field and percent of labeled cells per follicle in the areas of maximum labeling in liposome-[<sup>35</sup>S]DNA-treated skin histocultures were significantly higher than in naked-[<sup>35</sup>S]DNA-treated histocultures. High radioactive labeling by the [<sup>35</sup>S]DNA in the cell membranes and cell cytoplasm as well as in the cell nucleus was noted in the histocultured skin. In subsequent experiments, the Lac-Z gene coding for β-galactosidase was encapsulated in liposomes containing PC, cholesterol and phosphatidylethanolamine (PE). Incubation of the Lac-Z containing liposomes with mouse-skin histocultures demonstrated hair follicle targeting as demonstrated by staining with the substrate x-gal. These results demonstrate that an active gene can be targeted by liposomes to the hair follicle. Our results thus demonstrate that liposomes can target DNA into the hair follicle. This gives rise to the possibility of liposomes delivery of genes to the hair follicle that could alter hair pigment and growth of hair itself. The distinct advantage of liposome-based gene therapy of the hair-growth process is that liposomes specifically target the hair follicle.

- V-17** Modulation Of Differentiation Of Rat Tracheal Epithelial (RTE) Cells By Exogenous Matrix In Air Liquid Interface Cultures. E.A. Davenport and P. NETTESHEIM. Laboratory Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC 27709.

A pseudostratified mucociliary epithelium is observed after two weeks in culture when RTE cells are maintained on collagen I (col I) gel-coated membranes in air-liquid interface cultures. To determine the importance of exogenous extracellular matrix (ECM) for RTE cell growth and differentiation, RTE cells were seeded on uncoated or col I gel-coated membranes. Growth rate and plateau cell densities were similar under these two conditions; however, RTE cell attachment was typically 2-fold higher on col I gel-coated membranes. Cell distribution during log growth was significantly different, with numerous small colonies observed on gimsa-stained uncoated membranes and fewer, larger colonies observed on col I gel-coated membranes. Development of the secretory cell phenotype was delayed in the absence of exogenous ECM as determined by ELISA analysis of apical secretions utilizing antibodies that recognize a high molecular weight, hyaluronidase-resistant mucin-like glycoprotein and by quantitation of cells containing granules staining with Alcian Blue-Periodic Acid-Schiff's Reagent. Fifteen days after plating, mucociliary differentiation was limited on uncoated membranes with respect to mucin secretion and the development of preciliated/ciliated cells. In addition, Northern blot analysis demonstrated that in the absence of exogenous ECM, RTE cells express higher levels of collagen IV, thrombospondin, and fibronectin than on col I gel-coated membranes. These data suggest that col I gel is not essential for RTE cell growth or secretory cell differentiation; however, it increases cell attachment, accelerates

secretory cell differentiation, greatly enhances ciliated cell differentiation, and accelerates the down-regulation of ECM gene expression associated with differentiation.

- V-18** Regulation Of Tracheobronchial Mucous Cell Differentiation In Culture. REEN WU. California Regional Primate Research Center and Department of Internal Medicine, School of Medicine, University of California, Davis, CA 95616.

We have previously demonstrated that epithelial cells isolated from human and various animal airway tissues are able to express mucous cell differentiated functions, namely the synthesis and secretion of mucin, and the formation of mucous granules, in primary cultures. It was further found that the expression of mucous cell functions in culture can be regulated by a number of growth factors, calcium, vitamin A and extracellular matrix. Despite these studies, very little is known about the nature of these "mucogenic" factors in the regulation of mucous cell differentiation. In this communication, we carried out a cell kinetics study to illustrate the origin of mucous cell type in culture and the roles of vitamin A and TGF-β in the regulation of this differentiation. Dissociated human tracheobronchial epithelial cells were first plated on collagen gel substrata in the vitamin A-free, serum-free hormone-supplemented medium developed in our laboratory. At various times after plating, cultured cells were labeled with <sup>3</sup>H-thymidine, then chased in the medium supplemented with retinol and/or TGF-β. It was demonstrated that proliferative non-mucous cells are able to express mucous cell differentiation during the chase experiment. This expression is both vitamin A- and time-dependent. Furthermore, adding TGF-β at the time of retinol addition is able to inhibit the differentiation of these proliferative cell populations to mucous cell type. Using the RNA microinjection technique, we observed that this change in expression occurs at the genetic level. These results suggest that the mucous cell differentiation regulated by some of these mucogenic factors occurs at the gene expression level. (Supported by NIH grants.)

- V-19** Collagen Membrane Waffle Culture: Defining The Place Of Growth Modulators In Histokinetic Processes. J. Leighton. Aeron Biotechnology, San Leandro, CA 94577.

The three-dimensional structure of each normal tissue in nature must be of critical importance for durable function, since it is precisely maintained. The structure of a carcinoma resembles the tissue of its origin. The propagation of cancer tissue (Cancer Res. 29:2457, 1969) must also be a regulated process as increasing numbers of distinctive, similar assemblies of cells arise (Sci. 129:466, 1959). Secondary growths appear as recognizable variations of structural themes seen in the original tumor. An integrative approach to regulating such processes requires consideration of both the types of molecules associated with altering cellular processes, and of methods that reconstitute the physical conditions of nature including gradients and morphologic maturation.

tion and of diffusible metabolites, histophysiologic gradients (In Vitro 20:183, 1984). Furthermore, in nature there are no free edges to sheets of epithelium. Interepithelial tissue boundaries are numerous (In Vitro Cell. Dev. Biol. 28A:482, 1992). Some of these boundaries, common places for the beginning of cancer in nature, are easily established in gradient culture. Current developments in histophysiologic gradient culture methods use a thin irregularly transparent permeable sheet of collagen reinforced on one surface with a fine waffled, alveolated configuration of collagen. The convergence of simple 3-D gradient culture methods and the variety of growth modifying substances may place these molecules in a valid functional spatial context, effectively modifying abnormal tissue processes. Therapeutic alteration of the histologic structure of a carcinoma may alter the tumor's function in ways favorable to the health of the patient.

**JS-1** An Overview Of The *In Vitro* Approach To Blood-Brain Barrier Research: Benefits And Limitations. K.L. AUDUS and J.L. Holder. Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.

The endothelial cells lining the microvessels of the brain comprise a dynamic physical and metabolic barrier, the so-called blood-brain barrier (BBB). The BBB plays a vital role in tightly regulating the distribution of nutrients, drugs, and hormones between the blood and brain compartments. In a variety of disciplines, including physiology, pharmaceuticals, and endocrinology, a fundamental knowledge of the nature of BBB regulatory activity is of critical importance in understanding the effects of blood-borne substances on the brain. Consequently, in the last 20 years, a significant effort has been made to develop and characterize appropriate *in vitro* systems for studying BBB functions. Generally, these *in vitro* systems have had limited applications in reflecting the absolute permeability properties of the *in vivo* BBB. However, they have achieved significance in contributing information on basic cellular, molecular, and biochemical properties of the BBB. The purpose of this discussion will be to review the current state of *in vitro* models used in BBB research. (Supported by Hoffmann-LaRoche, Inc.)

**JS-2** Regulation Of Tight Junctions In Cultured Brain Microvessel Endothelial Cell Monolayers. T.J. RAUB. Drug Delivery Research, Upjohn Laboratories, The Upjohn Company, Kalamazoo, MI 49001.

Primary cultures of bovine brain microvessel endothelial cells (BMEC) provide a useful tool in evaluating transendothelial flux of molecules as a model for events at the blood-brain barrier (BBB). The validity of this system as a BBB model relies on the integrity of the cell monolayer and, thus, the formation of tight junctions, the expression of an authentic membrane recycling pathway involving vesicular traffic, and the subsequent development of membrane polarity. All of these cell biological issues have been the focus of intensive research during the past few years. The area that has received the most attention is the formation of tight junctions as these are very restrictive *in vivo* and are not represented fully *in vitro*. This presentation will focus on the regulation of tight junctions of BMEC grown on filters as measured by changes in transmonolayer electrical resistance and membrane-impermeant solute flux. We have been studying the tightening of BMEC monolayers by a yet -to-be identified glial cell factor(s) that works through a cyclic AMP-independent pathway. The role of second messenger systems in the control of tight junction was also examined using a pharmacological approach with various inhibitors and activators. The results suggested that BMEC tight junctions *in vitro* are positively affected by protein kinase C and elevated cAMP levels, and negatively influenced by elevated cyclic GMP levels and by inhibition of G protein.

**JS-3** Use of Collagen Gels to Study Properties of the Blood-Brain Barrier *In Vitro*. J.S. PACHTER and D. Biegel. Department of Physiology, University of Connecticut Health Center, Farmington, CT 06030.

Brain microvessel endothelial cells (BMEC), which comprise the physiological blood-brain barrier (BBB), can be isolated and cultivated *in vitro* under conditions where they retain much of their *in vivo* phenotype. To specifically study factors regulating the migration of leukocytes across the BBB, we have grown BMEC on hydrated collagen gels within Costar Transwell inserts. Use of the collagen gel or "plug" affords several advantages over direct plating onto membrane inserts: 1) It completely blocks the migration of BMEC through the membrane pores, thus ensuring that only a monolayer, and not multiple layers, of BMEC will develop; 2) It allows for the development of tighter endothelial junctions; 3) It enables invading cellular elements (e.g., leukocytes, pathogens) to be trapped and resolved from the BMEC monolayer, thus permitting quantitation of transendothelial migration; and 4) Other cell types can be grown within the collagen plug, underneath the BMEC monolayer, thus allowing for the study of interaction between the BBB and cells of the brain parenchyma. Results from preliminary studies will be presented detailing the morphologic and physiologic characteristics of BMEC grown in Costar Transwell inserts containing collagen plugs. Use of the Transwell/collagen plug system for evaluating leukocyte transendothelial migration will also be described.

**JS-4** Induction Of Blood-Brain Barrier GLUT1 Glucose Transporter By Brain-Derived Factors: Molecular Approaches Using Brain Endothelia And Astrocytes In Tissue Culture. W.M. PARDRIDGE. Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The blood-brain barrier (BBB) is comprised of the brain capillary endothelial cell *in vivo*. The unique biochemical characteristics of this cell are believed to be induced by factors secreted by brain cells such as astrocytes. The expression of the GLUT1 glucose transporter gene in brain is generally confined to the capillary endothelium, and this gene product is used as a marker of BBB tissue-specific gene expression. When brain capillary endothelial cells are grown in culture in the absence of astrocyte factors, there is marked down-regulation of both GLUT1 gene expression and the immunoreactive GLUT1 protein. GLUT1 gene expression is measured by nuclear run-on assays and mRNA levels determined with a quantitative polymerase chain reaction (PCR) assay. The immunoreactive GLUT1 protein is quantitated in tissue culture using a quantitative ELISA with human erythrocyte GLUT1 purified protein as an assay standard. There is evidence for a post-transcriptional regulation of GLUT1 mRNA translation efficiency and/or mRNA stability. The putative cis/trans mechanisms involving specific sequences within the untranslated region (UTR) of the GLUT1 mRNA and the cytosolic transacting factors are investigated with RNA gel shift assays, UV cross-linking studies, and RNase T1 mapping analyses. These studies show unique cis/trans regulatory mechanisms involving sequences within the 3'-UTR of the GLUT1 mRNA, and proteins present in both C6 rat glial cells or brain capillary endothelia.

- W-2** Methods And Systems For Automatic Environmental Control. T. Kozai and C. Kubota. Faculty of Horticulture, Chiba University, Matsudo, Chiba 271, Japan.

Measurement and control systems for monitoring and controlling the in vitro environment and photosynthetic growth and development of cultures are presented. The environmental factors discussed are mainly humidity, CO<sub>2</sub> concentration, light intensity and quality, air flow speed and temperature.

Methods for estimating the transpiration and CO<sub>2</sub> exchange rates (net photosynthetic and dark respiration rates) are presented. Benefits of a novel lighting system with diffusive optical fibers using light emitting diodes and/or a microwave-powered lamp as light source are discussed. Advantages and disadvantages of photoautotrophic micropropagation are presented.

Methods and systems for automatic environmental control in future micropropagation processes is proposed. Examples of the marked effects of physical environment on photosynthetic and non-photosynthetic growth, development and/or morphology of cultures are given.

- W-3** Somatic Coffee Embryo Quality Quantification Using Machine Vision. P.P. LING, Z. Cheng, and D.J. Musacchio. Bioresource Engineering Department, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903.

Somatic embryogenesis is a tissue culture technique that has the potential of generating genetically uniform plants and propagating disease-free plants in a large-scale mass production with fast turnaround. Automated, objective machine vision sensing techniques are being developed for quality evaluation of embryos regenerated from somatic embryogenesis processes. In this report, we will present the results of an effort in quantifying embryo's physiological development. Growth of somatic coffee embryos were quantified based on registered video images between maturation and germination stages. An image registration algorithm was implemented to register images of same embryos acquired at two different times. Growth features such as Elongation Coefficient and Growth Aspect Ratio were used to characterize somatic coffee embryo's physiological developments including elongation along apical axis and the ratio of longitudinal and lateral expansions along the apical axis of embryos. Based on the growth features, the machine vision system was able to predict embryo's viability for germination and consistently outperformed an operator's judgement. The germination prediction made by the machine vision system was based on training and took into account particular characteristics of a given embryogenesis prototype. The success shows a great potential of using the machine vision system for quality inspection for embryo production as well as a valuable sensing tool for embryogenesis study and prototype development.

- W-4** A Global Perspective On Automation Systems. M.A.L. Smith. 1021 Plant Sciences, University of Illinois, Urbana, IL 61801.

Innovations in automation for the micropropagation industry range from complex and expensive robotic maneuvers (for sterile transfer and plant manipulation) to simple, yet sophisticated devices for mechanizing routine procedures. In many cases, automated recognition devices (machine vision) are incorporated into the system design to minimize subjectivity. This presentation will highlight some recent trends towards cost-effective automation on an international scale.

- W-5** Micropropagation Automation Utilizing The Liquid/Membrane Approach. Roy E. Young. Agricultural and Biological Engineering Department, Clemson University, Clemson, SC 29634-0357.

Alternative techniques for plant micropropagation have evolved to a concept of liquid/membrane culture. Microporous, polypropylene membrane on liquid media emerged as an alternative support matrix. Equal or superior growth to that on conventional agar occurred for several species. The liquid/membrane concept also provided potentials for mechanization and functioned as a microbial barrier. Scale-up liquid bioreactors enabled automatic sensing and computer control of sucrose and dextrose medium composition. Growth of tobacco disc initiation cultures increased over agar with automated control of medium concentration. Nutrient depletion studies of watermelon tissues in liquid medium revealed that Ca<sup>++</sup> and K<sup>+</sup> reduced minimally over a 35-day growth period while NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> depleted significantly. Sugars remained high at 72.3% and 42.8% of initial levels for proliferation and elongation, respectively. Enhancements in growth rates in liquid/membrane culture have been observed with *Rex begonia* cv. Peacock, *Nicotiana tabacum* cv. Burley 21, and *Citrullus lanatus* cv. 'Charlee,' three species of orchids, rhododendron, cherry, tomato, hosta, fern, banana and sweet potato. Wire grid cutting devices have increased the cutting output over scalpel and forceps by a factor of 14. In a cut-and-dump protocol, this device became 4.8 times more productive for the total transfer process with both time and yield of viable bud clusters considered.

- W-6** Perfusion In A NASA Cell Culture System. G.F. SPAULDING. NASA/Johnson Space Center, Houston, TX 77058.

The NASA Biotechnology Program has developed a set of cell culture vessels that fundamentally changes the cell culture environment. Solid-body horizontal rotation with zero head space establishes a one-to-one viscous coupling of cell culture media with the vessel container, thereby eliminating boundary layers of gradient hydrodynamic shear. The elimination of gradient shear blocks the development of nucleation sites for turbulent eddies in media. Cell suspensions in solid-body rotation follow two major vectors: centrifugal generated by horizontal rotation and terminal acceleration in unit

gravity. Acceleration and rotational vectors lead to mechanical shearing effects that are predominantly derived from cellular movement of media, as opposed to media moving cells or mechanical stirrers moving cells. Consequently, this design imparts a cellular autoperfusion mechanism that is proportional to the cell/cell aggregate density and surface area. Autoperfusion of a cellular suspension facilitates the three-dimensional aggregation and growth leading to novel cell segregation. Experiment evidence supports numerical simulations. Cells cultured in a low hydrodynamic shear environment exhibit three-dimensional features and biochemical markers indicative of differentiation.

**W-7** Cell And Tissue Culture Research Within NASA Life Sciences. K. SCRIBNER. Martin Marietta Services, Inc., NASA Ames Research Center, Moffett Field, CA 94035.

Cell and tissue culture research at NASA Life Sciences focuses on the following areas: direct vs. indirect gravity sensing/response by individual cells and/or multicellular systems and the influences of gravity or microgravity on the regulation of genetic processes, signal transduction, membrane dynamics, intracellular transport, secretion, and cell-cell and cell-surface contacts in multicellular systems. This work is being advanced at several NASA centers and NASA-funded universities and research centers. Ground-based cell research has used models of cell cycle status and senescence to characterize the effects of gravity on appropriate transfer of growth factor signals in relation to the expression of growth regulatory genes/protooncogenes. Other work has focused on the role of mechanical stimulation on fibroblast cell responses to growth factors and the influences of gravitational force on biomineralization in a primary rat osteoblast-like cell line. Traditionally, space flight cell experiments have been performed in relatively simple, static cell culture systems and have demonstrated effects of gravity on cell growth and morphology, immunological responses, enzyme synthesis, circadian cycles, and cell differentiation in various eukaryotic and prokaryotic cell types. More complex cell culture systems are being explored that will allow testing of gravitational influences in short and long duration flight experiments utilizing three-dimensional, perfusion, or rotating systems for suspension and attachment cultures.

**W-8** Effects Of Shear On *In Vitro* Chondrogenesis. L.E. FREED<sup>1</sup>, G. Vunjak<sup>1</sup>, J.K. Blum<sup>1</sup>, J. Emmanuel<sup>2</sup>. <sup>1</sup>Massachusetts Institute of Technology, E25-342, Cambridge, MA 02139; <sup>2</sup>W.L. Gore, Flagstaff, AZ.

The effect of shear on *in vitro* chondrogenesis (cartilage formation) was studied using bioreactor cultivations of isolated cartilage cells (chondrocytes) on biodegradable polymer scaffolds. A no shear culture environment was established by fixing scaffolds within NASA-designed rotating bioreactors such that at steady state, the scaffolds moved at the same angular velocity

as the fluid phase. Low and high shear culture environments were established by fixing the scaffolds within flasks stirred at low and high rates. The biochemical and histological compositions of the resulting cell-polymer constructs were associated with shear rate as follows. Without shear, constructs consisted of mature chondrocytes, glycosaminoglycan (GAG) and collagen (types I & II). Shear induced the formation of an outer capsule that consisted of multiple layers of flat cells and type I collagen and represented up to 50% of the total implant volume. Shear increased the implant collagen content and decreased GAG content, per gram and per cell. Differentiated chondrocyte morphology and phenotype were best maintained when cell-polymer constructs were cultivated without shear.

**W-9** Cultured Human Endothelial And Smooth Muscle Cells. E.M. LEVINE, T.J. Goodwin, T.L. Prewett, and G.F. Spaulding. The Wistar Institute, Philadelphia, PA 19104; Johnson Space Center, Houston, TX 77058.

The human vasculature is a dispersed and complex tissue that plays an important role in physiologic homeostasis and is a significant factor in many pathologies. We pioneered the use of optimized culture media to sustain vigorous and long-term serial growth of human endothelial cells (ECs) and smooth muscle cells (SMCs). We showed that heparin (Hep) combined with fibroblast growth factors stimulates growth of both ECs and SMCs. ECs grown under these conditions express endothelial-specific characteristics such as Factor VIII-related antigen (FVIII:Rag) and angiotensin-I converting enzyme (ACE). However, the level of expression of both FVIII:Rag and ACE is markedly diminished with serial subculture. We found, however, that coculture with SMCs restores some of the lost expression of ACE and FVIII:Rag. The SMC phenotype is determined by the presence or absence of Hep in the medium. In the presence of Hep, matrix hyaluronic acid is upregulated as is  $\alpha$ -actin. On the other hand, downregulation of IL-1 $\alpha$  and collagen  $\alpha$ 1 (1) expression occurs. NASA has developed bioreactors for cell culture in which the gravity vector is randomized (thus simulating microgravity) and shear stress is very low. Because microgravity has been documented to affect the cardiovascular system in astronauts as well as the behavior of cultured leukocytes, we are planning to study the proliferative and phenotypic characteristics of cultured human vascular cells in the NASA bioreactor system.

**W-10** Culture Of Cardiac And Skeletal Muscle Cells In The NASA Bioreactor Vessel. N.A. Schroedl, R.E. Akins, G.R. Molnar, S.R. Gonda\*, and C.R. Hartzell. Nemour's Research Programs, Wilmington, DE 19899; \*Johnson Space Center, Houston, TX 77058.

Using the NASA designed High-Aspect-Ratio-Vessels (HARVs) and microcarrier beads, we have initiated a three-dimensional (3D) culturing system to characterize the effects of simulated microgravity on cultured cardiac and skeletal muscle cells. Cardiac cell cultures

contain 70-80% cardiomyocytes and 20-30% endothelial cells and fibroblasts. 2D-control and 3D-HARV cultures were inoculated at a density of  $1 \times 10^6$  cells/4.8 cm<sup>2</sup>/ml medium. After 3-6 days, cultures were assessed using scanning and transmission electron microscopy, fluorescence and Hoffman modulation contrast light microscopy, as well as functional assays. Cells in control cultures showed little 3D organization whereas cells in the HARV adhered to beads that aggregated into clusters containing 8-15 beads/cluster that exhibited distinct 3D layers: myocytes and fibroblasts appeared attached to the surfaces of beads and endothelial cells ensheathed the exterior structure. Although there was a difference in the dependence on aerobic pathways between control and HARV cells, enzyme-specific activities were the same in the two groups. Skeletal muscle satellite cells were plated onto Matrigel (diluted 1:100)-coated Nunclon dishes or beads at  $1 \times 10^6$  cells/55 cm<sup>2</sup>/10 ml medium. No significant difference was noted between the two culture systems, nor did total bead surface area (27.5, 55, or 110 cm<sup>2</sup>) effect the proliferative potential of the cells. Currently, the ability of satellite cells to differentiate and express muscle-specific proteins within the HARV is being tested.

**W-11** CEA Production In Human Colon Cancer. J. MILBURN JESSUP<sup>1</sup>, W. Fitzgerald<sup>2</sup>, J. Polanec<sup>1</sup>, R. Ford<sup>1</sup>, T. Goodwin<sup>3</sup>, G.F. Spaulding<sup>3</sup>, and D. Brown<sup>2</sup>. <sup>1</sup>Deaconess Hospital, Boston, MA 02215; <sup>2</sup>Krug Life Sciences, Houston TX 77058; <sup>3</sup>NASA/Johnson Space Center, Houston, TX 77058.

Malignant cells must implant, invade, and proliferate within an organ to create a metastasis. Recently, we have found that Carcinoembryonic Antigen (CEA), a 180 kD glycoprotein of the Ig supergene family, promotes colonization of nude mouse liver and lung when injected intravenously into nude mice prior to intrasplenic injection of  $2 \times 10^6$  poorly differentiated, human MIP-101 colon carcinoma cells. MIP-101 cells grown in monolayer culture or in the flanks of nude mice produce tumors without either CEA or metastases. MIP-101 cells implanted in the peritoneum produce local tumors, CEA, and liver and lung metastases. We tested whether the 3-D culture system of the NASA Rotating Wall Vessel (RWV) would support CEA production when MIP-101 cells were cultured on microcarrier beads. MIP-101 cells grew to densities of  $\sim 6 \times 10^6$  cells/ml from  $1-3 \times 10^5$  cells/ml over 7-10 days in GTSF or RPMI 1640 media with 10% FCS. MIP-101 cells produced low quantities of CEA (0.5 - 1.0 ng/ml) during the plateau phase of cultures. However, viability decreased once cells were cultured past 9 days associated with medium pH of 6.5 - 6.8. Glucose utilization decreased and cells came off microcarrier beads. O<sub>2</sub> tension was >80 mm Hg but PCO<sub>2</sub> was 80-120 mm Hg. MIP-101 cells from the RWV were tumorigenic but no more metastatic than monolayer culture cells. We conclude that 1) the RWV induces high metabolic activity in MIP-101 cells, 2) as culture senesces CEA is released into the medium, and 3) better pH control is needed.

**W-12** Growth And Gene Expression In Human Ovarian Cancer. J.L. BECKER<sup>1</sup>, T.L. Prewett<sup>2</sup>, T.J. Goodwin<sup>3</sup>, and G.F. Spaulding<sup>3</sup>. <sup>1</sup>Department of Obstetrics and Gynecology, University of South Florida, Tampa, FL 33606; <sup>2</sup>Krug Life Sciences, Houston, TX 77058; <sup>3</sup>NASA/Johnson Space Center, Houston, TX 77058.

Ovarian cancer is the leading cause of death from gynecologic malignancy. Difficulties in studying ovarian carcinoma are associated with the lack of adequate in vitro systems to sufficiently grow patient tumor cells in long-term culture. In order to gain a better understanding of factors that may contribute to the development of these aggressive tumors, we have established a model for ovarian tumor cell growth under three-dimensional conditions. Using the NASA Rotating-Wall Vessel, we have cultivated multicellular aggregates of ovarian tumor cells that exhibit unique morphological characteristics consistent with those properties expressed in vivo. We have used this model to observe growth characteristics achieved during extended three-dimensional culture in vitro and to examine expression patterns of protooncogenes, including erbB2 and ras, which are suggested to be associated with the progression of these tumors.

**W-13** Microgravity Affects Human Prostate Cancer Growth And Differentiation: A Preliminary Report. L.W.K. CHUNG<sup>1</sup>, T.J. Goodwin<sup>2</sup>, H.Y.E. Zhou<sup>1</sup>, T.L. Prewett<sup>2</sup>, G.F. Spaulding<sup>2</sup>, and S.M. Chang<sup>1</sup>. <sup>1</sup>U.T.M.D. Anderson Cancer Center - Urology Research Laboratory, Houston, TX 77030; <sup>2</sup>NASA/Johnson Space Center, Houston, TX 77058.

Our laboratories have been interested in elucidating molecular mechanisms of androgen action and the roles of stromal-epithelial interaction in prostate cancer growth and metastasis. These studies have led to the appreciation that factors elaborated by the host microenvironment (epigenetic) play a key role in prostate cancer growth and metastasis (Chung, L.W.K.; Cancer Biol. 4:183-192; 1993). In the present study, we embarked upon a preliminary study to characterize the effects of simulated microgravity environment on androgen-induced growth, glucose consumption, and gene expression by a human prostate cancer cell line, LNCaP. Results show the following: 1) The growth of LNCaP cells was induced linearly by 4-fold (between Day 3-9) upon the addition of DHT (100 ng/ml), when grown on the plastic dishes under serum-free condition. By contrast, DHT stimulated the growth of LNCaP cells in a cyclic manner when cultured under the microgravity conditions. At the term of the study (Day 14), DHT stimulated only marginally the increment of cell number ( $\sim 30\%$ ) when compared to ethanol-treated controls. 2) when LNCaP cells were cultured on plastic, DHT stimulated the production of prostate-specific antigen (PSA) mRNA and protein by 5- to 10-fold and 2- to 4-fold, respectively; PSA production was accelerated remarkably by DHT (20-fold, from 0.09 ng/ml/10<sup>3</sup> cells to 1.9 ng/10<sup>3</sup> cells) when cells were cultured under microgravity condition. 3) Microgravity induced marked alterations of the time-

course as well as the magnitude of androgen responsiveness in LNCaP cells. Microgravity research may add our understanding and appreciation of the effects of tumor microenvironment on prostate cancer growth and metastasis.

**W-14** Metabolic Activity Of Insect Cells Cultured In Simulated Microgravity. K. Francis<sup>1</sup>, N. Johnson<sup>1</sup>, K. O'CONNOR<sup>1</sup>, and G. Spaulding<sup>2</sup>. <sup>1</sup>Tulane University, New Orleans, LA 70118; <sup>2</sup>NASA/Johnson Space Center, Houston, TX 77058.

Insect cells are popular hosts for recombinant protein production because of the ease of cloning and expression in this system; however, their sensitivity to hydrodynamic forces impedes growth, particularly in large-scale vessels. A new process has been developed to culture insect cells in suspension for production of cell-derived biologicals: the cells are grown in simulated microgravity using the NASA High-Aspect Ratio Vessel (HARV). In simulated microgravity, hydrodynamic forces are greatly reduced, promoting cell growth and profoundly changing metabolic activity.

Based on our work to date with Fall Armyworm Ovary (Sf9) cells, we believe that the beneficial effects of the HARV are the greatest when the cells have a heightened sensitivity to hydrodynamic forces from an imposed stress. This occurs, for example, during stationary phase and viral infection. Consider the former, Sf9 cells grown in shaker flasks can be maintained in stationary phase for only 24 h before cell death becomes evident. When these cells are cultivated in the HARV, stationary phase can be extended for at least a week without any loss in viability. Moreover, these cells experience robust growth immediately upon dilution.

In the HARV, there is a redirection of metabolic energy from the repair of hydrodynamic damage to other cellular processes. To support this argument, we will present data on the rate of metabolite utilization/production for glucose, lactic acid, ammonia, and amino acids.

**W-15** Human Lymphocyte Locomotion In Randomized Gravity. N.R. PELLIS, T.J. Goodwin, D. Risin, B.W. McIntyre, R.P. Pizzini, D. Cooper, T.L. Prewett, and G.F. Spaulding. University of Texas M.D. Anderson Cancer Center, Houston, TX 77030; NASA/Johnson Space Center, Houston, TX 77058.

In long-term space travel, reduction in immunity suggests a possible direct effect of gravity on cellular responses. The effects of microgravity on human lymphocyte function were investigated *in vitro* using a culture vessel (Rotating Wall Vessel [RWV]) which randomizes gravitational vectors such that the net gravitational vector integrated over time approaches zero. In randomized gravity, lymphocytes did not locomote

through type I collagen, while static cultures supported normal movement into the collagen gel. The lymphocytes remain viable during the entire culture period but after 6 hours in the RWV and subsequent transfer to unit gravity (static culture), the lymphocytes are slow to recover locomotion into the collagen matrix. Loss of locomotion may be related to change in the state of lymphocyte activation. RWV culture did not significantly affect responses to polyclonal activation with phytohemagglutinin. When activated by anti-CD3 antibody and interleukin-2 (IL-2) prior to introduction to the RWV, the lymphocyte locomotory response was restored. Morphologically, lymphocytes from the RWV were rounded while locomotory lymphocytes are characteristically polar. Thus, in addition to the systemic stress factors that may affect immunity, isolated lymphocytes may respond to gravitational changes by ceasing locomotion through model interstitium. The RWV provides a model for the inhibition of lymphocyte locomotion in the microgravity of space. (Supported by the Gillson-Longenbaugh Foundation, Texas Advanced Technology Program, and NASA/JSC.)

- I-1001** Juvenile Hormone Production And Cell Proliferation By Cockroach Corpora Allata *In Vitro*. G.L. HOLBROOK, W.-H. Tsai\*, A.-S. Chiang\*, and C. Schal. Department of Entomology, North Carolina State University, Raleigh, NC 27695; and \*Institute of Life Sciences, National Tsing Hua University, Hsinchu, Taiwan 30043.

Insect corpora allata (CA) secrete juvenile hormone (JH), a humoral factor that regulates development and reproduction. Two mechanisms modulate *in vivo* JH production. First, neuropeptides released from neurohemal organs induce rapid elevation or reduction in JH biosynthesis. Second, slow alteration in number, size, and organelle content of CA cells modulates biosynthetic capacity of CA. Short-term *in vitro* assays lasting a few hours have been employed to examine rapid effects of neuropeptides on CA activity. However, a long-term assay in which CA retain biosynthetic or developmental capacity for an extended time period has yet to be established. It has been the purpose of our studies to develop an organ culture system for CA in order to facilitate elucidation of factors regulating slow, developmental changes in CA cells. We have found that in both short- and long-term assays, CA incubated in L15B medium produce significantly more JH than glands incubated in TC199, a medium frequently used in short-term assays. Maximal rates of JH biosynthesis are retained for at least 24 hours in L15B. Gland activity is significantly improved in TC199 augmented with amino acids or sugars suggesting that high levels of organic molecules are necessary for normal gland function. CA cells retain the capacity to undergo a proliferative cycle, as found *in vivo*, when glands are incubated in L15B with 10% FBS and 10% cockroach serum. Cockroach serum is essential for proliferation *in vitro* but the age, sex, or species from which the serum is obtained is unimportant. Since our recent work has shown that mitosis within the CA is under neural inhibition from the brain, we are now examining brain neuropeptides as regulators of cell proliferation.

- I-1002** Effect Of Long-Term Expansion And Maintenance Of *Spodoptera frugiperda* (Sf9) Insect Cells In Bioreactors On Expression Of Recombinant Cyclo-Oxygenase-2 Following Infection With A Baculovirus. A.T. NAHAPETIAN, J.T. Pepe, M.B. Covington, and H.J. George. The Du Pont Merck Pharmaceutical Co., Glenolden, PA 19036.

Baculovirus infected insect cell systems have been used for expression of a number of recombinant proteins in many short-term, but few long-term, productions. Aims of the present study were: 1) to produce milligram quantities of a recombinant human cyclo-oxygenase-2 for drug discovery, and 2) to test the effect of Sf9 cell passage number on the recombinant protein expression in a long-term semi-continuous production. Basal medium used was TNM-FH medium (10% fetal bovine serum, 4 mM L-glutamine and 0.1% Pluronic-F68). Baculovirus used was *Autographa californica* nuclear polyhedrosis virus which was engineered for production

of human cyclo-oxygenase-2. A CelliGen bioreactor (3.4 L working volume) was used for continuous expansion and maintenance of stock Sf9 cells. Every three days, 2.4 - 2.5 L of the stock culture was harvested and culture volume was made-up to 3.4 L with the basal medium. Harvested cells were resuspended in fresh medium and following infection (MOI=3) with 100 ml of a baculovirus suspension they were transferred into either a small (1.3 L working volume) or a larger (3.4 L working volume) CelliGen bioreactor. The infected cultures were monitored for cell number, pH, metabolites, and cyclo-oxygenase-2 activity and they were harvested on Day 3 post infection. Thirty-five productions were completed within a 171 day period. The enzyme activity was found to be lower for the productions in 1.3 L bioreactor (0 - 180000 units) than for those performed in the larger vessel (127500 - 600000 units), most probably due to higher cell density and limitation of nutrients in the former environment. In addition, total cyclo-oxygenase-2 activity per harvest increased with increasing Sf9 cell passage number most probably due to either adaptation and higher metabolic rate for the infected cells or selection for those with higher anabolic/lower catabolic activity for the recombinant protein.

- I-1003** Replication Of *Choristoneura biennis* Entomopoxvirus In *Choristoneura fumiferana* (Lepidoptera: Tortricidae) Cell Lines. S.S. SOHI, B.M. Arif, B.J. Cook, J.A. MacDonald, and G.F. Caputo. Forest Pest Management Institute, Natural Resources Canada, P.O. Box 490, Sault Ste. Marie, Ontario P6A 5M7, Canada.

We are interested in developing *Choristoneura biennis* entomopoxvirus (CbEPV) as an expression vector, and also in exploiting this virus for insect control. For this we need a cell line permissive to this virus. Over the years we have developed many cell lines from the tissues of forest insects. Several of these lines (IPRI-CF-1, -5, -8, -10, -16T, -124T, and IPRI-MD-66; FPMI-CF-22, -27, -33, -34, -41, -70, and FPMI-MS-5) were tested for permissiveness to CbEPV. Hemolymph from *C. fumiferana* larvae infected with CbEPV was used as virus inoculum. The virus was allowed to adsorb for 1 hour and then incubated at 20° C. The inoculated cells were examined with a light microscope for the presence of EPV spheroids in the cytoplasm. Three of the cell lines (CF-16T, CF-27, and CF-34) produced EPV spheroids, confirming virus replication. About 50% of the cells of CF-16T and CF-27 lines produced spheroids, but only 10% of CF-34 cells did so. Electron microscopy of CF-16T cells infected with the EPV showed that almost all the cells replicated the virus, although only about 50% of the cells had spheroids. EPV replication in cells grown in serum-free EX-CELL 400 medium was as good as in cells grown in Grace's medium with 10% FBS. Work is in progress to test spheroids produced in cell culture for infectivity to the host larvae.

- I-1004 *In Vitro* Primary Cell Culture Of *Hermisenda crassicornis* Neurons. C.T. Tamse<sup>1</sup>, A.M. KUZIRIAN<sup>1</sup>, P.J.S. Smith<sup>2</sup>, and C. Collin<sup>3</sup>. <sup>1</sup>*Hermisenda* Resource Laboratory, <sup>2</sup>National Vibrating Probe Facility, Marine Biological Laboratory, Woods Hole, MA 02543. <sup>3</sup>Laboratory of Adaptive Systems, NINDS/NIH, Bethesda, MD 20892.
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The marine nudibranch, *Hermisenda crassicornis*, has been an important model in the study of associative learning and memory. The numerous published works include *in situ* studies on the morphological, electrophysiological, cellular, and molecular aspects of its nervous system. However, *in situ* CNS preparations may not always provide in-depth information on mechanisms of cell-cell interactions, synapse formation, or construction of neural circuits. To make these neural mechanisms accessible for study, we isolated and cultured neurons from identified regions of the *Hermisenda* CNS. Dissociated cells grew in modified Leibovitz L-15 medium and were maintained for up to 18 days without changing the culture medium. The morphological characteristics of viable neurons are described. The eye and pedal neurons (LP1, 2, & 3) which are involved in phototactic circuitry were also successfully isolated and grown. Electrophysiological measurements of cultured LP1 cells showed membrane characteristics similar to those previously reported in CNS preparations *in situ*. This newly developed, primary neuronal culture method is reproducible, allows for experimental manipulations and analyses, and will help provide insights into the precise relationships between specific neurons and behavioral changes.

- P-1001** High Efficiency Of Regeneration Of Peanut Using A Nonimbibed Immature Leaflet Culture Method. S.D. UTOMO, A.K. Weissinger, H.T. Stalker, and T.G. Isleib. Dept. of Crop Science, North Carolina State University, Raleigh, NC 27695.

Efficiency of plant regeneration from immature leaflets of peanut (*Arachis hypogaea* L.) was compared among several explant treatments in an effort to maximize recovery of plants from cultures. Explants were derived from either dry, mature seeds or mature seeds imbibed in water for 1 or 4 days. To avoid confounding of treatment effects with variation among individual seeds, both nonimbibed and imbibed leaflets originated from a single seed. For each seed, four nonimbibed leaflets from one leaf were excised, sterilized, rinsed, and plated on MS-based medium amended with 4 and 2 mg/l BAP and NAA. The embryo axis with the other leaf (four remaining leaflets) along with one cotyledon attached were then allowed to imbibe water for 1 or 4 days. Leaflets were then harvested, sterilized, and plated. After 4 weeks in culture, 53% of nonimbibed leaflets and 37 and 6% of leaflets imbibed for 1 and 4 days produced shoots. Subsequently, regeneration efficiency was compared among immature leaflet cultures from nonimbibed mature seeds of four peanut varieties from 3 diverse botanical types—2 subsp. *hypogaea* var. *hypogaea* (NC 7 and NC Ac 17092, 1 subsp. *fastigiata* var. *fastigiata* (NC Ac 17090), and 1 subsp. *fastigiata* var. *vulgaris* (PI 262000). Response averaged from 53% (NC 7 and NC Ac 17092) to 9% (NC Ac 17090 at 4 wk after plating and from 61% (NC 7 and NC Ac 17092) to 27% (NC Ac 17090) at 6 wk. The response of var. *vulgaris* fell between the other varieties. Shoot proliferation was significantly greater in var. *hypogaea* than in other varieties, while shoot proliferation in var. *fastigiata* was significantly lower than var. *vulgaris*. These results suggest that subsp. *hypogaea* can be cultured efficiently from nonimbibed immature leaflets.

- P-1002** Adventitious Shoot Regeneration From Cotyledons, Leaf Tissue, And Cell Suspension In Hackberry (*Celtis Occidentalis* L.). Zong-Ming Cheng and Nian-Qing Shi. Department of Horticulture and Forestry, North Dakota State University, Fargo, ND 58105.

Adventitious shoots were successfully regenerated from cotyledons, leaf tissue on modified MS (Murashige and Skoog, 1962) media in hackberry (*Celtis Occidentalis* L.). Forty-four percent of the cotyledonary explants regenerated an average of 2.7 shoots per explant on the medium supplemented with 15  $\mu$ M BA and 2.5  $\mu$ M IBA. Fifty-five percent of the leaf explants regenerated an average of 3.0 shoots per explant on the media containing 10  $\mu$ M BA. Shoots were also successfully regenerated from cell suspension cultures. Callus were used to initiate the cell suspensions in MS liquid medium containing 15  $\mu$ M BA and 5  $\mu$ M IBA. After 1 month in culture, cell suspensions were grown in MS medium supplemented with 2.5  $\mu$ M 2,4-D for 4 weeks and the cultures were transferred to fresh medium weekly. The cell cultures were then plated on regeneration media and approxi-

mately 80% of the cultures were transferred biweekly. Shoot formation occurred from the remaining 20% of the cultures without any transfer on the medium supplemented with 17.5  $\mu$ M BA and 5  $\mu$ M IBA in 2 months. The regenerated shoots were elongated on MS medium supplemented with 3.0  $\mu$ M BA. Rooting of the regenerated shoots were proven very difficult and further studies were underway.

- P-1003** Tissue Culture Of Ginkgo. N.D. CAMPER, R.J. Keese, and D.E. Wedge. Clemson University, Department of Plant Pathology and Physiology and Department of Forest Resources, Clemson, SC 29634-0377.

Ginkgo, *Ginkgo biloba*, leaves produce secondary compounds such as flavones, glycosides, ginkgolides, and other diterpenes. Specifically, ginkgolides show significant pharmacological activity for treating vascular disorders by inhibiting platelet aggregation. However, extraction procedures are lengthy and in vitro yields are low. Plant cell and tissue culture of ginkgo may facilitate selection of plant lines as well as production and extraction of these secondary metabolites. Ginkgo seeds were collected in December from two trees on the Clemson campus and the fleshy seed coat was removed. Stony seed coats were removed and the inner seedcoat was surface sterilized. Explants evaluated include intact embryos, cotyledons, radicles, and endosperm. Murashige and Skoog Minimal Organics media was supplemented with various combinations of 2,4-D, NAA, BA, and kinetin. Once a detection and selection system for lines with increased secondary compound production is developed tissue culture could be used to produce source material for commercial production.

- P-1004** *In Vitro* Culture Of Scots Pine (*Pinus sylvestris* 'Ladoga') From Dormant Bud Explants. T. Pheh and K. PRUSKI. Alberta Tree Nursery & Horticulture Centre, R.R. #6, Edmonton, Alberta, Canada T5B 4K3.

Micropropagation of Scots pine was investigated over a two-year period. Vegetative, terminal buds from mature trees were used as explants. Each year buds were collected from early December to April. Following sterilization in alcohol and 0.5% bleach, scales of the buds were removed. The tips of exposed dormant candles were removed. Candles were then placed on modified Quoirin & LePoivre (LP) media with various concentrations of BAP and sucrose. Explants were incubated at 20° C with 16 h photoperiod at 3000 lux. A BAP level of 3 mg/L and sucrose at 5% concentration were optimal for growth of cultures. The time of explant collection did not have any effect on culture growth. However, there was a difference in response between tree selections.

Cultures were transferred onto fresh media every 3 weeks. Short needles grew from fascicles in 3 to 4 weeks. This was immediately followed by interfoliage fascicle bud development in 7 to 8 weeks. At this stage cultures ceased growth and all attempts to get the explants into the next stage, namely shoot elongation, have failed. So far, different concentrations of media

with various levels of IBA, GA<sub>3</sub>, and GA<sub>4+7</sub> have been tested. The experiments will continue since the development of a micropropagation protocol for pine (from vegetative buds of mature trees) will be very useful in propagating this valuable species.

- P-1005** Microcuttings Of *Taxus x Media* cv. *Hicksii*. R.M. CERDEIRA, J.D. McChesney, and C. Burandt, Jr. Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS 38677.

Taxol, a diterpene with exceptional anticancer activity, occurs as a secondary compound in several *Taxus* species. *Taxus* is a very slow growing tree or shrub and traditionally has been propagated by cuttings. Tissue culture have been a useful tool for propagation of many slow growing plants and may be important for propagation of high taxol yielding plants. Different salt media varying in salt concentrations were evaluated for bud breaking. On Hoagland salts supplemented with 1.0 mg/L of kinetin and 20 g/L of sucrose shoot were induced and growth was maintained. Reducing salt concentration caused the shoots to root.

- P-1006** *In Vitro* Germination Of *Musa velutina* Seeds. N. PANCHOLI, A. Wetten, and P.D.S. Caligari. Department of Agricultural Botany, School of Plant Sciences, University of Reading, Reading RG6 2AS, UK.

In the genus *Musa*, germination is extremely variable and relatively difficult. Even more difficulties are faced when producing hybrids. The seed yield of hybrids in breeding programs is usually low and often, to ensure the viability and survival of seeds, it is necessary to attempt to germinate a large excess of these seeds. In this context, *in vitro* embryo culture is an invaluable tool for obtaining desirable hybrid plants in a short time. Seeds of *Musa velutina* were sown in seed trays in a peat based mixture. The *in vivo* seed germination reached 78% but only after 9 months. Because of this delayed and intermittent germination, a new sample of embryos were excised and inoculated on media with 1/2-strength MS salts, with or without supplementation with various concentrations of gibberellic acid (GA<sub>3</sub>). Light and dark conditions were also used to test their effect on embryo germination. After 2 weeks, 82% of embryos germinated in the dark with 0.1 μM GA<sub>3</sub>. Addition of GA<sub>3</sub> increased the shoot length and root number over the non-GA<sub>3</sub> containing treatment. Similarly, dark conditions gave a significant increase over light conditions for all the parameters except root number where light or dark conditions did not make any difference. Thus the present study highlights the importance of various components of the *in vitro* culture of *Musa* embryos and the advantage over direct use of greenhouse-sown seeds both in terms of the time taken to germinate and the final percentage.

- P-1007** Influence Of A Blue-Green Algal Extract On Shoot Multiplication And Rooting Of *Vitis rotundifolia*. AIDA M. ALLAM. Dept. of Horticulture, Agric. College, Menufiya University, EGYPT.

The effects of a blue-green algal extract on *in vitro* cultures were evaluated in muscadine grape (*Vitis rotundifolia* cv. 'Summit') shoot proliferation and rooting cultures. In shoot multiplication studies, water extracts of dried, ground algae were incorporated into a modified Murashige and Skoog (MS) medium with 2.25 mg/l IBA. Algal extracts (0, 2%, 4% [w/v]) were added to the media at the rate of 20 ml per liter. Media containing the algal extracts influenced culture fresh weight accumulation. Compared to controls, cultures exhibited 141% and 120% greater growth on media supplemented with 2% and 4% extract, respectively. Enhanced shoot proliferation and development were obtained when media were supplemented with algal extract. An increased number of both small (<2cm) and long (>2cm) shoots was obtained. In rooting studies, extract was similarly incorporated (0, 2%, 4%, 6%) into a rooting media containing 1.02 mg/l IBA. Roots were classified on a scale of one to four. One represented no roots; four represented vigorous, thick (over 2 mm), long (over 20 mm) roots with numerous laterals. Root quality increased with the addition of algal extract, with an increased number of larger, more vigorous roots. Mean rooting values increased from 1.9 in the control, to reach 2.4 - 2.5 with extract.

- P-1008** Screening Of *Zea mays* Plants For Phosphinothricin Resistance Using The Chlorophenol Red Test. M.S. Wright, R.D. Shillito, K. Launis, C. Bowman, M. Hill, and J. DiMaio. Ciba Seeds, Research Triangle Park, NC 27709.

Culture of leaf pieces on medium containing the pH indicator, chlorophenol (CR) accurately predicts whether plants are transgenic for the Bar gene. This gene confers resistance to phosphinothricin (PPT), the active ingredient in the herbicide, Basta®. A concentration of 1 mg/l of PPT or Basta® (a.i.) is generally sufficient to determine whether the plant is transgenic. Within 4 days, transgenic leaf pieces acidify the medium which changes color from deep red to bright yellow. Some genotype specificity in the level of PPT required is observed. We used this method to screen over 30,000 putative T<sub>0</sub> transformants and found that 2% were Bar expressing transgenics. We also used this screen to evaluate the progeny of the 176 and 171 transgenics previously reported by Koziel et al., 1993. The 176 transgenic plants contained the Bt and the Bar gene; the 171 transgenic plants contained the Bt, the Bar, and the GUS gene. Positive results in the CR test correlated 88% with insect kill data. Correlation data with PCR and GUS analyses will be discussed.

**P-1009** Production Of Rice Resistant To AHAS-Inhibiting Herbicides. T.P. CROUGHAN. Rice Research Station, Louisiana Agricultural Experiment Station, L.S.U. Agricultural Center, Crowley, LA 70527.

Red rice is a weed that causes significant losses in southern U.S. rice production. Providing an effective method for controlling red rice during the rice crop would help alleviate these losses and also provide southern rice growers with the option of growing rice every year instead of rotating with other crops to control red rice. The project described in this report was directed towards developing herbicide-resistant rice germplasm as a possible solution to the red rice problem. Rice lines with herbicide resistance have been developed through conventional and tissue culture procedures. The lines are resistant to herbicides that inhibit the AHAS enzyme system. However, the mechanism of resistance in these lines does not appear to be due to a resistant AHAS enzyme. The genetics of the resistance is currently being determined. Replicated field trials with the earliest line developed indicated that herbicide treatment reduced yield of the herbicide-resistant line by an average of 10%, while non-resistant rice yields were reduced an average of 98%. Crosses among the herbicide-resistant lines and with other varieties have been made, and another culture is being conducted on the resulting hybrids to rapidly develop homozygous offspring.

**P-1010** Use Of Culture Filtrates Of *Discula destructiva* To Develop Resistance In *Cornus florida*. D.E. WEDGE and F.H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Flowering dogwood (*Cornus florida*) is an important landscape and understory forest tree in the southeastern United States. Dogwood anthracnose (DA) is a severe disease of dogwoods, ravaging native populations at higher elevations. Since its discovery, DA has moved southward down the Appalachian mountains from the northeast and in some locations every dogwood tree has been killed. Toxins from culture filtrate (CF) of the causative fungus (*Discula destructiva*) have been fractionated, chemically characterized, and evaluated in bioassays. Strong evidence exists that toxins are involved in the injury to dogwood tissues during infections and colonization. Established callus cultures have been challenged with a stepwise exposure to sublethal concentrations of culture filtrates of isolates of *D. destructiva*. A single clonal line of *C. florida* was selected, evaluated, and compared with that of control tissue. Specifically, toxin-containing partially purified culture filtrates (PPCFs) are being used as a selection tool coupled with somaclonal variation to develop callus resistant to the DA fungus and its associated toxins. The advantage of PPCFs is that selection is broad based, avoiding counter coevolutionary phenomena by the pathogen, and thus should be stable over a long period of time. Once a selection and detection protocol is developed, a large population of dogwoods will be screened for resistance.

**P-1011** Phenotypic Segregation (Herbicide Tolerance) Analysis In Elite Inbred Transgenic Maize. P. BULLOCK, M. Galatowitsch, D. Foster, K. Newhouse, C. Lewnau, K. Cook, S. Bagnall, J. Chojecki, V. Guerin, M. Wilson, S. Jiao, J. Register, I. Evans, K. Wang, M. Alphas, and T. Friend. ICI Seeds, Slater, IA 50244.

Friable non-organized embryogenic callus (type III) of an elite stiff stalk inbred maize line was bombarded (Biolistic PDS-1000) with a construct conferring tolerance to the herbicide "Ignite". Transgenic clones were isolated (Bialaphos selection) and transgenic plants representing unique transformation events were crossed two times to non-transgenic plants of the same inbred to create families (pseudo BC1) containing null and hemizygous plants. In addition, transgenic progeny representing BC1S1, BC1S2 and a partial diallel made among different transformation events (utilizing BC1 parents) were developed. Replicated herbicide field trials (U.S. and France: 0, 400, 800, 1600 G.A.I./Hectare, "Ignite" for BC1 progeny) and greenhouse screening trials (7500 G.A.I./Hectare, "Ignite") indicates that both Mendelian and non-Mendelian phenotypic segregation were observed. Transgene expression in both hemizygous and homozygous families is discussed. Somaclonal mutants (pale green and striate) were observed at the BC 1S2 stage and appeared to segregate for herbicide tolerance.

**P-1012** Reduction Of Ionic Mercury By Transgenic Plants. H.D WILDE<sup>1</sup>, N.M. Stack<sup>2</sup>, L.V. Azarraga<sup>1</sup>, and R.B. Meagher<sup>2</sup>. <sup>1</sup>Environmental Research Laboratory, U.S. EPA, Athens, GA 30605; <sup>2</sup>Genetics Department, University of Georgia, Athens, GA 30602.

Bacteria can oxidize and reduce metals in reactions that result in metal immobilization and/or detoxification. This research examined the transfer of metal redox capability from bacteria to plants. In mercury-resistant bacteria, metal detoxification is catalyzed by the *merA* gene product, mercuric ion reductase. The nucleotide sequence of *merA* was changed by PCR-based mutagenesis to improve gene expression in plants. The construct pMER2, in which the modified *merA* gene was placed behind the CaMV 35S promoter, was introduced into *Arabidopsis thaliana* by *Agrobacterium*-mediated transformation. *merA* mRNA was detected in pMER2-transformed plants by RT-PCR. Seeds from transgenic plants germinated on agar-solidified medium containing 60  $\mu\text{M}$   $\text{HgCl}_2$ , a level lethal to seeds from nontransformed plants. In the presence of 20  $\mu\text{M}$   $\text{HgCl}_2$ , nontransformed seeds germinated, but did not produce roots or true leaves. Transgenic seedlings exposed to 20  $\mu\text{M}$   $\text{HgCl}_2$  developed normally and flowered. The amount of metallic mercury ( $\text{Hg}^0$ ) produced by transgenic plants is being measured using a mercury vapor analyzer. The potential for using this approach to develop plants for heavy metal bioremediation will be discussed.

- P-1013** Transformation Experiments In White Spruce. R. WHETTEN<sup>1</sup>, K. Cheah<sup>1</sup>, C. Loopstra<sup>1</sup>, D. Ellis<sup>2</sup>, C. Lanz-Garcia<sup>1</sup>, and R. Sederoff<sup>1</sup>. <sup>1</sup>Department of Forestry, North Carolina State University, Raleigh, NC 27695-8008; <sup>2</sup>Department of Horticulture, University of Wisconsin, Madison, WI 53706.

Molecular genetic analysis of cell and tissue differentiation in conifers has been slow because of the paucity of stable transformation techniques that allow regeneration of transgenic individuals of this economically and ecologically important group. We have used recent advances in transformation of white spruce (*Picea glauca*) embryogenic callus to establish three independent callus lines transgenic for constructs containing a loblolly pine promoter fused to the GUS reporter gene. Plantlets regenerated from these lines show different patterns of GUS staining, suggesting that position effects and/or epigenetic variation play an important role in determining the tissue specificity of transgene expression.

We have also explored methods for improving the efficiency of the spruce transformation procedure, so that multiple independent transformation events can more readily be generated and analyzed. We have analyzed the requirements for *Agrobacterium* infection of spruce embryogenic callus and tested alternatives to kanamycin for selection of stably transformed callus lines. We have also tested the use of luciferase as a reporter gene that can be assayed nondestructively to allow segregation of nontransformed from transformed cells without exposure to antibiotics or herbicides. Incremental improvements in many steps of transformation protocols should result in a significant increase in the overall transformation efficiency.

- P-1014** Transformation Of Peanut With *Bt* Crystal Protein *CryIA* (c) Gene And The Nucleoprotein Gene Of Tomato Spotted Wilt Virus (TSWV). CHONG SINGSIT, W.A. Anderson, M.T. Adang, and P. Ozias-Akins. Department of Horticulture, University of Georgia, P.O. Box 748, Tifton, GA 31793-0748.

The redesigned *Bacillus thuringiensis* crystal protein *cryIA* (c) gene and a nucleoprotein gene of tomato spotted wilt virus (TSWV) (from D. Gonsalves, Cornell) were successfully introduced into peanut using microprojectile bombardment. The *Bt* gene is under the control of CaMV 35S promoter. The 3.4 kb redesigned *Bt* gene cassette has been directly cloned into Bgl II digested plant transformation vectors, pH602 and pSG 3525. The TSWV nucleoprotein gene was excised from pBin19-pecNP1 by a partial digestion with *HindIII* (since the NP gene contains internal *HindIII* site) and ligated into the plant transformation vector pCB13. These vectors contain hygromycin phosphotransferase (*hph*) gene conferring resistance to antibiotic hygromycin. Immature peanut seeds were used to initiate embryogenic callus cultures that were used in bombardment experiments. Three peanut cultivars, Florunner, MARC-1, and Toalson, were used in seven bombardment experiments with plasmid constructs containing the redesigned *Bt* gene and three with plasmid containing the TSWV gene.

DNA from the embryogenic callus lines resistant to hygromycin showed the presence of *hph*, *Bt* and TSWV genes by PCR. Based on our previous experience all or most of these PCR positive callus lines should also be Southern positive. Regeneration of plants and ELISA immunoassay of the proteins are underway.

- P-1015** Optimization Of Silicon Carbide Fiber-Mediated DNA Delivery Into Regenerable Sorghum And Maize Tissue Cultures. HEIDI F. KAEPLER<sup>1</sup>, Jeff F. Pedersen<sup>1</sup>, and David A. Somers<sup>2</sup>. <sup>1</sup>USDA-ARS, Lincoln, NE 68583; <sup>2</sup>University of Minnesota, St. Paul, MN 55113.

Silicon carbide fiber-mediated DNA delivery, an inexpensive, simple, nonproprietary transgene delivery method, has been documented to result in stable transformation of nonregenerable maize. Optimization of DNA delivery parameters for silicon carbide fiber-mediated DNA delivery into *regenerable* sorghum and maize tissue cultures has resulted in increased frequencies of DNA delivery to levels which should be sufficient for selection of transformants. Using modified parameters, DNA encoding the *uidA* (GUS) and *bar* genes was delivered into regenerable callus cultures of maize (A188 x B73) and sorghum via silicon carbide fibers. The frequency of cells observed transiently expressing GUS following treatment and histochemical staining was approximately 10-fold higher than that observed when nonmodified parameters were used. The frequencies of DNA delivery obtained via modified silicon carbide fiber-mediated DNA delivery is of the same order of magnitude as that obtained via microprojectile bombardment of similar regenerable tissues. Sorghum and maize calli treated with pBARGUS and silicon carbide fibers have been placed in selection experiments to determine if transgenic plants can be recovered from cells into which DNA has been transferred via silicon carbide fibers.

- P-1016** Sunflower (*Helianthus annuus* L.) Transformation Via Particle Bombardment. ALEXANDER GAPONENKO<sup>1,2</sup> and John Finer<sup>2</sup>. <sup>1</sup>Center of "Bio-engineering" Academy Sciences of Russia, Moscow; <sup>2</sup>Ohio Agricultural and Development Center, The Ohio State University, Wooster, OH 44691.

Different *in vitro* regeneration protocols have been compared and evaluated for use in establishing an efficient sunflower transformation system. We have defined the conditions necessary to obtain hundreds of sunflower plants from cultured immature embryos, cotyledons, and hypocotyl segments. Regeneration protocols are genotype independent. Evaluation of different bombardment parameters (distance, pressure, prechamber volume, type and size of particles), culture conditions (explant size, time of preculture, different media) and two different promoter constructions (35SGUS and RTL2GUS) have yielded high levels of transient expression of the GUS gene in bombarded immature embryos. The average number of blue spots routinely obtained under optimal condition was 5000 per bombardment, or 200 spots per immature embryo. GUS positive shoots have been obtained using particle bombardment of immature embryos.

- P-1017** Cultivar-Independent Transformation And Regeneration Of Carnation Using *Agrobacterium tumefaciens*. E. FIROOZABADY, Y. Moy, W. Tucker, and K. Robinson. DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608.

We have developed an efficient method for transformation and regeneration of plants from different cultivars of carnation, *Dianthus caryophyllus* L. This method is based on a step-wise selection and a secondary regeneration/multiplication process which eliminate chimerism and produce fully transformed shoots. Whole leaves from *in vitro* shoot cultures were mixed with *A. tumefaciens*, cocultivated for five days and selected on chlorsulfuron to produce transgenic shoots. All of the regenerated shoots were vitrified. These were normalized, rooted and transferred to the greenhouse. 100% of regenerated plants were transformed based on rooting assay, GUS assay, PCR and Southern. Geneticin (G418) and kanamycin used for selection, or other explants used as a source for inoculation were not as efficient for transformation. The leaf transformation method has facilitated introduction of desirable genes into different cultivars of carnation.

- P-1018** Microprojectile Bombardment Prior To Co-Cultivation With *Agrobacterium* Improves GUS Expression In Watermelon Cotyledons. M.E. COMPTON<sup>1</sup>, D.J. Gray<sup>1</sup>, E. Hiebert<sup>2</sup>, and C.M. Lin<sup>2</sup>. <sup>1</sup>CFREC, University of Florida, 5336 University Avenue, Leesburg, FL 34748 and <sup>2</sup>Plant Pathology Dept., 1453 Fifield Bldg., University of Florida, Gainesville, FL 32611.

Cotyledons from 6-day-old watermelon (cv. Minilee) seedlings were cultured for one day on shoot regeneration medium (modified MS medium with [per liter] 30 g sucrose, 0.1 g myo-inositol, 5  $\mu$ M BA and 7 g TC agar; SRM) before subjecting them to bombardment with DNA-coated tungsten particles, infection with *Agrobacterium*, or a modified procedure where explants were bombarded with tungsten particles before co-cultivation with *Agrobacterium*. Treated cotyledons were placed in X-gluc solution 72 hours after bombardment or transfer to selection medium (SRM with 100 mg/l kanamycin and 500 mg/l Mefoxin®). GUS expression was estimated 24 hours after transfer to X-gluc. The number of blue foci was 1.6- to 2.7-fold greater for explants bombarded with tungsten particles before infection with *Agrobacterium* than for those treated with *Agrobacterium* alone or bombarded with DNA-coated tungsten particles, respectively. From 400 to 1000 GUS-expressing colonies were observed per plate when watermelon cotyledons were wounded with microprojectiles before infection with *Agrobacterium*.

- P-1019** Delivery Of Proteins And DNA Into Intact Plant Cells. F.-S. Wu, A.B. CAHOON, and M. Shulleeta. Department of Biology, Virginia Commonwealth University, Richmond, VA 23284.

It is highly desirable to develop a simple, efficient, inexpensive, and widely applicable method for delivering macromolecules into intact plant cells. We think that the

difficulty at present in direct gene transfer of intact cells is not caused by the presence of the cell wall itself, but by the absence of DNA molecules on the plasmalemma. We have found that osmotic changes which create void spaces between the cell wall and plasmalemma will allow the macromolecules to pass through the cell wall, maximizing the contact of macromolecules with the plasmalemma. A simple electroporation can then be used to effectively transfer the macromolecules into the plant cells. Onion epidermal peels were used to examine the effects of plasmolysis on cell viability and permeability to protein and DNA molecules during electroporation. The cytoplasmic streaming was also visualized in these cells. It was found that proteins could pass through the cell wall at a statistically significant higher rate when living plasmolyzed cells were used ( $22.05 \pm 6.05\%$ ) than when ethanol-permeabilized cells were used ( $5.69 \pm 2.97\%$ ). When plasmolyzed cells were electroporated at various field strengths, it was found that they were much more tolerant to the electroporation than those normal, unplasmolyzed cells. Using freshly sectioned tobacco stems, we have also observed that: plasmid containing a  $\beta$ -glucuronidase gene could pass through the cell wall and be introduced into the cells by electroporation.

- P-1020** Donor Chromosome Elimination In Asymmetric Somatic Hybrids Of Nicotiana: Effect Of Radiation Dose And Time In Culture. H.T. TRICK and G.W. Bates. Department of Biological Sciences, Florida State University, Tallahassee, FL 32306.

The production of asymmetric somatic hybrids could be a useful method for the transfer of discrete traits between plant species if conditions can be found to efficiently eliminate most of the genome of the donor species but yet retain the trait of interest. In order to reliably produce highly asymmetric hybrids, it is necessary to understand the factors underlying donor-chromosome elimination. We have examined the effects of radiation dose and time in culture on the elimination of donor genetic material in donor-recipient fusions between *Nicotiana plumbaginifolia* and *N. tabacum*. Mesophyll protoplasts of a kanamycin-resistant line of *N. plumbaginifolia* were gamma-irradiated and fused with mesophyll protoplasts of *N. tabacum* bearing the sulfur (Su) mutation (recessive genotype is yellow). Hybrid calli were recovered by selection on media containing kanamycin and were analyzed for their retention of genetic material of the donor, *N. plumbaginifolia*. In one set of experiments, the extent of chromosome elimination was assessed by dot-blot hybridization using a *N. plumbaginifolia*-specific repetitive DNA sequence as a probe. In a second set of experiments, DNA elimination was assessed by scoring the fraction of hybrid calli that were able to green (i.e. that retain wild type allele of the donor that complements the Su mutation in the recipient). Both assays showed that the elimination of donor DNA increased as the radiation dose applied to the donor was increased, over the range of 5 to 50 krad. In addition, donor DNA continued to be eliminated for the first 12 months in culture. At the highest dose (50 krad),

our data indicate that about one-third of the hybrids have lost 85% or more of the donor genome, which represents the retention of three, or fewer, donor chromosomes.

**P-1021** Regeneration Of Sunflower (*Helianthus annuus* L.) From Mature Cotyledons. C. BAKER, N. Fernández, and C. Carter. Plant Science Department, Northern Plains Biostress Laboratory, Box 2140-C, South Dakota State University, Brookings, SD 57007.

The lack of an efficient regeneration method limits the application of transformation techniques for the improvement of sunflower (*Helianthus annuus* L.). The method must be reproducible with a variety of genotypes, have high efficiency, and produce high number of shoots per explant. Mature cotyledons from germinated seedlings appear to be the most responsive explant. The age of the seedling before the cotyledons are removed is an important factor, with older cotyledons having a reduced regeneration potential. Other factors which have affected both efficiency of regeneration and number of shoots per cotyledons were plant growth regulator concentrations and types, silver nitrate, explant size and orientation and genotype. Shoots have been obtained and have been rooted.

**P-1022** Tetraphenylboron And Phenylboronic Acid Induce Somatic Embryogenesis. J. Ponsamuel and P. Dayanandan. Plant Tissue Culture Unit, Madras Christian College, Madras 600 059, INDIA. Current address: Plant Biology Division, The Samuel Robert's Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Tetraphenylboron (TPB) and Phenylboronic acid (PBOA) induce somatic embryogenesis independently and also in combination with either Benzylaminopurine (BAP) or coconut milk (CM) from the cotyledonary explants of tea (*Camellia sinensis*), peanut (*Arachis hypogaea* cv. JL-24), and from the leaf segments of country potato (*Coleus parviflorus* cv. CP-9). TPB and PBOA at a concentration of 10  $\mu$ M induced embryogenic callus and embryoids in peanut and tea. In combination with 10  $\mu$ M BAP or with 10% CM both compounds induced embryoids from the leaf derived callus of country potato. PBOA induced ~75% more explants than TPB in all the three species tested. Somatic embryos were produced 20-40 days after the callus initiation. The embryogenic potential was maintained by secondary embryogenesis in the subculture stages through successive generation of embryoids. Although  $\alpha$ -Naphthaleneacetic acid (NAA) in peanut and 2,4-Dichlorophenoxyacetic acid in tea differentiates embryoids, the plant conversion rate was only 2% as compared to 35% in PBOA and 20% in TPB induced embryoids. In country potato, TPB and PBOA induced embryoids showed 90 and 98% conversion rate respectively, whereas NAA induced embryoids had a low conversion frequency of 20%. Embryoids from all these species developed into plantlets when cultured on auxin free MS medium for about 30 to 40 days and were capable of greenhouse survival. We propose that these compounds

may be efficient in not only triggering somatic embryogenesis in plants, but also enhance the frequency of embryoid to plant conversion ratio.

**P-1023** Thidiazuron Mediated *In Vitro* Regeneration Of Peanut Plants. M. KANYAND, A. Porobo Dessai, and C.S. Prakash. Tuskegee University, Plant Molecular Genetics Lab, School of Agriculture, Tuskegee, AL 36088.

High-frequency induction of multiple shoots was achieved in valencia peanut (*Arachis hypogaea* L.) cv. New Mexico. Zygotic embryos excised from mature seeds were germinated on the basal Murashige-Skoog (MS) medium, and resulting plants were used as a source of explants. Various parts of the peanut plant (except the root) produced multiple shoot primordia and shoots when cultured on MS medium with thidiazuron (TDZ) (0.5, 10 or 30 mg/l). The hypocotyl and cotyledon explants were relatively more responsive to organogenesis than leaf, petiole or internodal explants as they produced more shoots (15-20 shoots per explant). Hypocotyl explants with shoot primordia when separated into smaller clumps and cultured on TDZ medium, continued to produce new adventitious shoots. Shoots rooted normally on a basal MS medium and developed into healthy and fertile plants when transferred to soil. The TDZ-mediated *in vitro* regeneration system is rapid and repetitive, and may be useful in gene transfer research for the development of transgenic peanut plants.

**P-1024** In Vitro Systems For Plant Regeneration Of Switchgrass. K. ALEXANDROVA, P.D. Denchev, and B.V. Conger. Department of Plant and Soil Science, University of Knoxville, TN 37901-1071.

A highly efficient system for *in vitro* plant regeneration of switchgrass has been developed. Plants were regenerated from two cultivars 'Alamo' and 'Cave-in-Rock' after using mature caryopses and leaf tissue from young seedlings or secondary tillers of greenhouse or *in vitro* grown plants as explants. Regeneration was by both embryogenesis and organogenesis. A different regeneration response was obtained with two auxins (2,4-D and Picloram). The best results with picloram were obtained with 22.3  $\mu$ M plus 15.0  $\mu$ M BAP. A narrower response was obtained with 2,4-D. One thousand regenerated plants from *in vitro* cultures were established in the field. Node cultures were used for fast clonal propagation. Five tillers of each plant were harvested in the 4-5 node stage. The nodes were sterilized, split longitudinally and planted on MS medium supplemented with 1 mg/l BAP. In 2 months, between 2 and 5 shoots developed from a single node. These also had well developed roots and could easily be established in pots. Therefore, using this system for clonal propagation more than 40 plants could be produced from one greenhouse grown plant in a 3-month period. Approximately 30% of the tested plants produced either shoots or inflorescences from node cultures. Inflorescences appeared only from the uppermost node. They do not occur

from nodes of flowering plants. These results suggest that the inflorescences may already be in an early stage of development at the time of explanting nodes.

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**P-1025** Somatic Embryogenesis In Rapid Cycling (*Brassica rapa*). R. BANKS, M.R. Uddin, G. Small, and M.I. Shafi. Division of Science and Mathematics, Rust College, Holly Springs, MS 38635.

This study concerns with generation of somatic clone of rapid cycling brassica (*Brassica rapa*) using tissue culture technology. Induction of adventitious shoot production and somatic embryogenesis was attempted using cotyledon explant. Combination of Benzyl 6-aminopurine (BAP) and Naphthalene-acetic-acid (NAA) were effective in inciting a morphogenic response from cotyledon explant. On some treatment occasional adventitious shoot initiation was also observed. BAP or NAA alone were ineffective in eliciting morphogenic response. Callus production was achieved either with 2,4-D alone or in combination with BAP. Semi-friable, transparent and fast growing calli were produced by a combination of 2,4-D and BAP. Subculturing these calli on liquid media resulted in the production of early staged somatic embryos.

**P-1026** A Rapid And Repetitive Somatic Embryogenesis System In Sweetpotato. Q. Zheng, A. Porobo Dessai, and C.S. Prakash. Plant Molecular Genetics Lab, School of Agriculture, Tuskegee University, Tuskegee, AL 36088.

Sweetpotato (*Ipomoea batatas*) leaf and petiole explants from genotype PI 318846-3 were cultured on MS medium containing a factorial combination of auxins and cytokinins at various levels. Embryogenic calli were observed on explants cultured in the dark on a medium with 2,4-D (2.5 mg/l) and BAP (0.25 mg/l). These embryogenic explants when transferred to a basal MS medium and cultured in light, developed distinct somatic embryos which exhibited characteristic developmental stages upon further culture. Addition of ABA at 2.5 mg/l enhanced the development of somatic embryos. Somatic embryos were observed 4 weeks after the initiation of explants into culture. The secondary somatic embryo production was achieved by the culture of primary somatic embryos on MS medium containing various levels of 2,4-D. When cultured on a basal MS media, somatic embryos germinated into full, normal plants. The somatic embryogenesis system is rapid, repetitive and highly proliferative; thus, it may be of potential use in the development of transgenic sweetpotato plants especially with the particle bombardment technique.

**P-1027** Cryopreservation Of Embryonic Axes Of Hazelnut (*Corylus avellana* L. cv. Barcelona). B.M. REED<sup>1</sup> and M.N. Normah<sup>2</sup>. <sup>1</sup>U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521; <sup>2</sup>Department of Botany, Faculty of Life Sciences, University Kebangsaan Malaysia, 43600 Ukm Bangi, Selangor Darul Ehsan, Malaysia.

Long-term storage of large seeded nuts such as hazelnut is not possible at present due to loss of viability after about one year. Cryopreservation of the seed or embryonic axes would provide a safe germplasm storage method. Isolated embryonic axes of freshly harvested hazelnut (*Corylus avellana* L. cv. Barcelona) were successfully cryopreserved by drying either under laminar flow or over silica gel to 8% to 15% moisture content. Natural inhibition of seed germination in hazelnut complicated study of the axes. Culture of untreated axes from freshly harvested nuts on NCGR-COR medium with or without growth regulators resulted in 85% viability and 77% shoot growth. High viability of both control and cryopreserved axes was maintained after 2 to 8 weeks storage. Shoot production in control axes initially was near 50% but declined to about 20% within one month. Changes in the culture medium did not overcome inhibition due to storage. Since seed germination of hazelnuts is high before they drop from the tree and decreases rapidly thereafter, this method may be most useful for nuts gathered and processed at or before normal harvest.

**P-1028** Anther Culture Studies In Pigeonpea. P. VIJAYAKUMARI and S. Narasimha Chary. Cytogenetics and Tissue Culture Laboratory, Department of Botany, Osmania University, Hyderabad-500 007, India.

Embryoids and embryogenic callus could be induced from anthers of *Cajanus cajan* (L.) mill sp. Anthers cultured on medium containing N<sub>6</sub> inorganic salts and MS organic salts supplemented with 2,4-D and BAP. Sucrose level was varied between 3-10% with 0.8% of agar and 0.2% of charcoal. The embryoids derived were further differentiated into haploid plants. Mid-uninucleate stage of anthers proved to be suitable for anther culture in terms of embryogenic callus and embryoids formation. 4% of sucrose was most effective. Pretreatment of buds at 4° C for 10 days showed increase in and embryoids formation. Cytological studies revealed the presence of haploid chromosome number. Anatomical and SEM studies showed the various stages of embryogenesis and further differentiation. These studies show the effect of temperature, sucrose and hormones which has significant role on embryogenesis.

- P-1029** Somatic Embryogenesis And Plant Regeneration In Chickpea (*Cicer arietinum* L.). A.K. Sudha Vani and V.D. Reddy. C.P.M.B., Osmania University, Hyderabad-500 007, A.P., India.

Chickpea being a recalcitrant species various protocols were tried for efficient regeneration. Direct regeneration from epicotyl explant in cv. JG-62 was 100% with 5-6 shoots/explant on B5 media supplemented with 1.0 mg/l BAP + 1.0 mg/l KN and 0.5 mg/l IAA and from leaves (37.08%) on B5 media with 10.0 mg/l BAP + 1.0 mg/l NAA which were rooted on B5 media with 4.0 mg/l IAA + 0.5 mg/l KN. Differentiation of shoots (2.42%) was achieved from hypocotyl derived callus of JG-62 on B5 media supplemented with 1.0 mg/l BAP. Whereas C-235 exhibited shoot bud formation (1.5%) and PGC1 failed to regenerate. Somatic embryogenesis was achieved in cotyledon derived cultures of cv. JG-62 on Gamborg's basal medium 2.0 mg/l 2,4-D and 3% sucrose (46.4%). An increased frequency was observed on media supplemented with L-glutamine (56.6) and L-asparagine (48%) in addition to 2.0 mg/l 2,4-D. The embryogenic callus was yellow, smooth and nodular. Anatomical and SEM studies of which revealed the presence of proembryonic masses, globular and early torpedo shape embryoids. Stages further to torpedo was never observed. Immature embryos of cv. JG-62 cultured on B5 media with 3.0 mg/l Zeatin and 0.04 mg/l IAA gave 5-6 embryolike structures (ELS) (66.6%). Histological studies of which revealed two distinct zones, a meristematic zone and embryogenic zone. Plantlets from these structures were obtained on B5 media + 0.5 mg/l BAP. Thus factors like genotype, media and explant play an important role in regeneration suggesting the obscure molecular nature of morphogenetic competence.

- P-1030** Induction Of Somatic Embryos In Suspension Cultures And Plant Regeneration Of Indica Rice. T. Usha Rani and G.M. Reddy. Center for Plant Molecular Biology, Osmania University, Hyderabad-500 007, AP, India.

Mature embryos of Rasi, Tellahamsa and Getu were cultured on MS medium with 2.5 mg/l 2,4-D, 0.5 mg/l KN and 3% sucrose which gave 92.5% primary callus. Callus was subcultured on same medium supplemented with 2% sorbitol for proliferation. Visual selection of nonfriable, nodular, pale yellow to white type a highly embryogenic calli (1 gm) were transferred to AA medium with 2.5 mg/l 2,4-D, 0.5 mg/l KN, 10 mM proline and 3% maltose. By specific duration and dilution ratio (cell suspension: fresh medium) of cultures, mostly globular shaped embryoids (50-60) were recovered. Development of these embryoids was promoted by their transfer to a medium containing 1 mg/l IAA, 2 mg/l BAP, 0.6 mg/l KN with 3% sucrose and later developed into normal green plants. Tellahamsa was superior (67%) in embryoid production followed by Getu and Rasi, 58 and 53%, respectively. Direct embryoid production can be exploited in genetic transformation and transgenesis through protoplasts.

- P-1031** *In Vitro* Development Of An Interspecific (*O. Sativa* x *O. Longistaminata*) Hybrid And Cytological Status Of Regenerated Hybrid Plant. NUZHAT FATIMA, S.Y. Anwar, and T.P. Reddy. Department of Genetics, Osmania University, Hyderabad-500 007, India.

*Oryza longistaminata* with (AA) genome representing the primary gene pool, having the desirable trait of open floral characteristic that promotes out pollination, does not cross normally with the cultivated rice *Oryza sativa* (AA genome). Therefore, it is essential to use both *in vivo* and *in vitro* techniques to stimulate pollination and to nurture the otherwise abortion prone embryos to form a mature Fi hybrid. In the present study, *O. sativa* was used as female and *O. longistaminata* as a pollinator. Seven-day-old interspecific hybrid embryos of *O. sativa* x *O. longistaminata* were excised and cultured on LS medium supplemented with 1 mg/l 2,4-D and 0.5 mg/l Kinetin. Hybrid plant regeneration was observed on half strength LS medium with 0.01-0.02 mg/l IAA and 0.04 mg/l Kinetin. Cytological studies of the regenerated hybrid plant showed diploid ploidy level with  $2n=24$ .

- P-1032** Plantlet Regeneration-Influence Of Genotype, Explant Age And Hormones On Rhizogenesis In Safflower. (*Carthamus tinctorius* L.). TEJOVATHI GUDIPATI and R.R. Das. SOS in Botany, Jiwaji University, Gwalior 474 011, India.

Cotyledons and apical buds from 4-, 5-, and 6-day-old seedlings of Mangira, S-4, A-1, and APRR-3 were cultured on B5 medium supplemented with various levels of BAP (6-benzyl aminopurine). The multiple shoots obtained were transferred to Basal medium for a week. Later the shoots were inoculated on B5 medium with different levels of NAA ( $\alpha$ -Naphthaleneacetic acid) (1-3 mg/l) for rooting. About 15-25% regeneration of complete plantlets was achieved from different varieties. The paper reports the influence of genotype, age of the explant, and hormone level on regeneration.

- P-1033** Regeneration Of Cucumber (*Cucumis sativus* L.) Plants From Excised Cotyledon. ANUP K. MISRA and S.P. Bhatnagar. Department of Botany, University of Delhi, Delhi-110 007, India.

Cucumber (*Cucumis sativus* L.), a member of the family Cucurbitaceae, is an important food crop in India as well as in many other parts of the world. Being an economically important crop the application of *in vitro* technique is of great value. A simple and efficient system for regenerating plants from cotyledonary explants can be useful in somaclonal variation and genetic transformation studies.

A protocol has been developed that is comprised of the following steps: 1) direct shoot regeneration from cotyledonary explants, 2) shoot elongation and rooting, and 3) transplantation. Cotyledon explants of various ages from seedlings were cultured on MS medium supplemented with different combinations of growth hormones. High frequency shoot regeneration was induced from 1- and 3-day-old cotyledons cultured on MS

medium supplemented with 10  $\mu$ M and 5  $\mu$ M BAP, respectively. Elongated shoots (on MS basal medium) rooted on 1  $\mu$ M IBA supplemented medium. The plantlets were transplanted in plastic pots containing garden soil and sand mixture (1:1). After two weeks, these plants were transferred to field with 80% survival rate. These plants produced normal flowers and fruits. Histological studies will be discussed.

**P-1034** Occurrence of Developmental Abnormalities in Callus Cultures of *Eruca sativa*. NEBU JOHN and Amla Batra. Lab No. 5, Botany Department, University of Rajasthan, Jaipur 302004, India.

*Eruca sativa* Mill. is an oil seed crop belonging to the family Brassicaceae and is very extensively cultivated in North Western regions of India. It thrives well under both rainfed and drought conditions. Due to the presence of strong incompatibility barriers with other *Brassica* species, direct use of *E. sativa* in breeding program has been limited. Callus was initiated from hypocotyl explants on Murashige & Skoog (MS) medium supplemented with 2,4-D (2 mg/l). Subsequent transfer of the callus to the same basal medium incorporated with BAP (4 mg/l) led to the formation of embryogenic callus. Along with the normal mature embryoids, leafy appendages of different shapes were also seen in the cultures. More than 50% of the somatic embryoids also revealed developmental aberrations on rooting medium (i.e. 1/2 strength MS medium without any growth hormones). Thus, mature embryoids instead of giving rise to roots, produced multiple shoots of either normal growth or abnormal growth. The calli kept the morphogenetical potential only up to 3 passages of 3 week intervals. However, greening and nodulation could be retained in the subsequent passages.

**P-1035** Transfer And Expression Of T-DNA Into Rice And Sorghum Via *Agrobacterium*. R.H. SMITH, T.S. Ko, and S.H. Park. Dept. Soil and Crop Sci., Texas A&M University, College Station, TX 77843.

Shoot apices of *Oryza sativa* cv Maybelle, and *Sorghum bicolor* cvs RTX430 and BTx3197 were explants for cocultivation and gene transfer using *Agrobacterium tumefaciens*. The plasmid, pGUS3 (35S promoter driving expression of GUS and Nos promoter driving the NPTII) and plasmid, pAct1-bar (actin promoter driving the bar gene) were both used in sorghum studies; the latter was used in rice. Apices were isolated at 4 d and cultured on MS salts with 20 g/l sucrose and 0.1 mg/l kinetin. Two days later they were cocultivated with *Agrobacterium* +/- acetosyringone for 2-3 days. Apices were transferred to MS salts plus 250 mg/l clavamox +/- 1 ppm glufosinate ammonium (ppt). Rooted plants were transferred to soil and self-pollinated. PCR analysis of primary sorghum, RTX430, plants showed 58 out of 80 plants positive for NPTII and GUS. One was positive for GUS and NPTII in southern analysis. Sorghum primary plants exposed to the bar gene showed varying leaf damage from Ignite. Maybelle shoot apices cocultivated with *Agrobacterium* containing the bar gene

were selected on 0.5 mg/l ppt and surviving apices grown to maturity. A southern blot of one primary plant showed the bar gene was transferred. Analysis of progeny is in progress, and inheritance patterns and molecular analysis will be evaluated.

**P-1036** Development Of Herbicide Resistant Turfgrass Through Mutant Selection And Protoplast Transformation. LISALEE, Cynthia Laramore, Peter Day, and Nilgun Tumer. AgBiotech Cente, Rutgers University, NJ 08903.

Protoplasts were isolated from embryogenic suspension cultures callus cultures and regenerated on feeder layer from a turfgrass suspension culture of creeping bentgrass. High osmolarity with 5% mannitol was used for the isolation, initial divisions and regrowth of protoplast. Six creeping bentgrass varieties produced protoplast derived calli. Plants were regenerated from SR1020, South shore, and Pennlinks. *E. coli*  $\beta$ -glucuronidase (GUS) gene constructs were used to quantitate direct DNA uptake in protoplasts. Transient expression of the GUS gene was obtained using either polyethylene glycol (PEG) or electroporation. We are in the process of introducing AHAS gene into creeping bentgrass using protoplast transformation. In addition, we are using mutant selection to obtain Pursuit resistant creeping bentgrass. Mutant selection provides the advantage of producing herbicide resistant varieties from non-recombinant DNA technology, eliminating the regulatory barriers to commercialization. Toxic concentrations of Pursuit were determined by performing kill curves on embryogenic callus and suspension cultures. We used 20  $\mu$ M Pursuit for mutant selection with a number of callus cultures. Cultures with good growth were periodically transferred to fresh media with the same concentration of herbicide. Plants were regenerated from several tissue sectors with vigorous growth and herbicide spray tests of these are in progress.

**P-1037** Transformation Of Caucasian Bluestem (*Bothriocloa caucasica*, L.) - An Apomictic Forage Grass. J.O. Ponsamuel, A. Trieu, D.V. Huhman, and C.I. Franklin. Plant Biology Division, The Samuel Robert's Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Caucasian bluestem (*Bothriocloa caucasica*, L.) is well acclimatized to the climatic and the edaphic conditions of the south central U.S. Further improvement of this forage grass through conventional breeding is difficult due to its apomictic nature. The low protein content brings down the nutritive quality of this fodder grass. Genetic manipulation to enhance the nutritive value of this grass species is of great practical value. The forage quality of caucasian bluestem (CBS) may be improved by introducing gene(s) coding for specific storage protein(s). As a first step towards this objective, we have been successful in developing a transformation system for CBS via particle bombardment. We have optimized conditions for DNA delivery into CBS embryogenic callus using the plasmid pBARGUS. Stable integration of the reporter gene in the transformed CBS plants was con-

firmed by the Southern blot analysis. In transient GUS assays the plasmid pAct1-D, containing rice actin promoter-intron-GUS fusion, showed at least twice the number of GUS expressing cells than pBARGUS. Since pAct1-D lacks a selectable marker, we cloned the bar gene driven by 35S promoter contained in pBARGUS into pAct1-D to construct PNF93-1. Using this construct, we have regenerated CBS plants showing bialaphos resistance. Attempts are now in progress to introduce the soybean vegetative storage protein genes (*vspA* or *vspB*) as well as the 15 kDa *zein* gene from corn driven by rice actin promoter into CBS.

**P-1038** Rapid Production Of Transgenic Corn By Microprojectile Bombardment Of High Type II Immature Embryos. Andy S. Wang, Eduardo Brambila, and ROSE A. EVANS. Novel Genetics Department, Northrup King Co. Research Center, Stanton, MN 55018-4308.

A rapid transformation system for a high Type II inbred line is outlined. 255 10-day-old (1.0 to 1.5 mm long) immature embryos were isolated from high Type II corn and bombarded with tungsten particles coated with plasmid DNA containing PAT (phosphinothricin-N-acetyltransferase) and GUS (-glucuronidase) genes. The bombarded embryos were transferred to a bialaphos containing medium 16 hours later. 61 of the embryos produced enough callus to assay for GUS activity after one month. Eleven of them were GUS positive. Seventeen plants were regenerated on a bialaphos containing medium from 5 of these GUS positive calli, grown in a greenhouse, and pollinated with pollen from normal high Type II plants. Seven of the pollinated plants produced seed. Overall, 24% (61/255) of the bombarded embryos produced bialaphos resistant calli, 4.3% (11/255) were also GUS positive. Six months elapsed between embryo isolation for bombardment and transgenic seed harvest. This system is ideal for studying shooting pattern, damage to biological materials, transient expression, and stable transformation frequency.

**P-1039** Optimization Of PEG-Mediated Stable Transformation For Regenerable Maize Protoplast Cultures. ANDY S. WANG, Rose A. Evans, and Jeff L. Rosichan. Novel Genetics Department, Northrup King Co. Research Center, Stanton, MN 55018-4308.

An efficient polyethylene glycol-(PEG)-mediated transformation system has been developed for regenerable maize protoplast cultures. Age of suspension cultures, protoplast yield and growth rate, nurse culture conditions, DNA buffer and concentration, PEG concentration and treatment period, and selection initiation date are all important in the transformation system. An ideal suspension culture is less than one-year-old with a mitotic index of 10%, a protoplast yield of  $1 \times 10^6$  protoplasts per gram of fresh culture, and a plating efficiency of 0.1%. Protoplasts grown on either one-week-old B73 or BMS nurse culture had approximately 1.0% plating efficiency and formed callus culture in two weeks. Transformation was carried out in 20% PEG for

30 min. Plasmid DNA containing PAT and GUS was dissolved in water and 50  $\mu\text{g}$  of the DNA per  $1 \times 10^6$  protoplasts were used in each treatment. Addition of demethylating agent (5-azacytidine) increased transgene expression. Under optimum culture and PEG conditions, up to 35 PAT stable transformants per  $1 \times 10^6$  protoplasts were produced. 50% of the transformants also were positive for the GUS gene. The PEG method had a much higher transformation frequency than electroporation with our maize protoplasts.

**P-1040** Transient GUS Expression In Lily Bulb Scales And Cell Suspensions Transformed By Particle Bombardment. JOSÉ M. PEÑAFIEL and Kathryn Kamo. Floral & Nursery Plants Research Unit, USDA, USNA, Beltsville, MD 20705-2350.

Regenerable bulb scales and cell suspensions of Asiatic lily (*Lilium elegans*) and the interspecific hybrid lily (*Lilium elegans* x *Lilium longiflorum*) were bombarded with gold particles coated with pBI505 and pACT1-D plasmids, which contained the uidA gene for  $\beta$ -glucuronidase (GUS) expression, using the biolistic particle gun. Murashige & Skoog (1962) supplemented with 3 mg/l kinetin and 0.5 mg/l naphthalene acetic acid for bulb scales, and 2 mg/l dicamba for cell suspensions were effective for producing cell and tissue growth that expressed GUS. The optimum settings, for the Biorad biolistic PDS-1000/He system particle gun were 1800 psi shooting pressure and 1 inch shooting distance. The time for optimal GUS expression was 5 days after shooting.

Phosphinothricin (5 mg/l) in the culture medium inhibited plant regeneration in both bulb scales and cell suspensions.

**P-1041** Effects Of Concentration Of Acetosyringone And *Agrobacterium tumefaciens* On GUS Gene Transformation Efficiency Of *Populus*. F.H. HUANG and X.Y. LI. Department of Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701.

High frequency GUS gene transformation of a *Populus* hybrid NC-5331 leaf explant has been found to significantly depend upon the concentrations of both the bacterium and Acetosyringone (AS), the inducer of virulence gene in Ti-plasmid. At a bacterial suspension diluted to one in 25 and with no AS added prior to incubation with leaf segments, the transformation rate (22%) was higher than other treatments with AS at 25, 50, 75 or 100  $\mu\text{m}$ . However, at a bacterial dilution of one to 50, transformation rates were 51% and 30% with AS at 25 and 50  $\mu\text{m}$ , respectively. These rates were significantly higher than these with AS at 0, 75, and 100  $\mu\text{m}$ . The selective medium with Kanamycin and histological assays of GUS gene transformation showed the calli and plantlets have been transformed.

- P-1042** Stable Transformation of Long Day Photoperiod-Adapted Soybean Somatic Embryogenic Culture. L.-N. TIAN<sup>1,2</sup>, D.C.W. Brown<sup>2</sup>, H. Voldeng<sup>2</sup>, and J. Webb<sup>1</sup>. <sup>1</sup>Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6; <sup>2</sup>Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

High latitude-adapted soybean cultivars and germplasms are unique for their daylight insensitivity or adaption to long day photoperiods. To date, there is no report of genetic transformation for these germplasm types. Embryogenic suspension cultures of long day photoperiod-adapted soybean, cv. Nattawa, were bombarded with tungsten particles coated with DNA containing  $\beta$ -glucuronidase (GUS) and hygromycin resistance genes. The bombarded cultures were selected by two ways: direct selection with hygromycin at 35 mg/L or 50 mg/L, or step-wise selection from 20-50 mg/L. No cultures survived when selected directly at high levels of hygromycin. However, step-wise selection gave rise to survival in bombarded cultures. The majority of selected cultures showed GUS expression. These cultures have been expressing GUS activity for six months. PCR analysis showed these cultures are positive for the GUS gene. Plants are being recovered from these cultures. This is the first report of stable transformation of long day photoperiod-adapted soybean cultures and extends the previous reports of transformation via bombardment of embryogenic suspension cultures in other types of soybean germplasms.

- P-1043** The Role Of DNA Methylation In The Expression Of The Dc8-GUS Transgene In Carrot (*Daucus carota* L.). YUANGXIANG ZHOU<sup>1</sup>, J.M. Magill<sup>2</sup>, C.W. Magill<sup>2</sup>, and R.J. Newton<sup>1</sup>. Departments of Forest Science<sup>1</sup> and Plant Pathology & Microbiology<sup>2</sup>, Texas A&M University, College Station, TX 77843.

DNA methylation has been linked to gene activity in differentiating and developing plant tissues. The objective was to determine the involvement of methylation in the expression of a gene transferred into carrot (*Daucus carota* L.) tissues by particle bombardment. The expression of the Dc8-GUS gene construct in calli and somatic embryos in response to treatments of 5-azacytidine (5-azaC), an inhibitor of DNA methylation, was investigated by histochemical assay of GUS. Compared to nontreated controls, the 5-azaC treatment increased Dc8-driven GUS expression in both calli and somatic embryos. The increase occurred with treatment either to *E. coli* with the plasmid insert before bombardment or to the carrot tissues after bombardment and plasmid transfer. GUS expression, increased by the 5-azaC treatment, was enhanced by ABA treatment of both calli and somatic embryos and was more prominent in the latter. Decreased digestion of the 5-azaC-treated plasmid DNA with the restriction enzyme EcoRII (specific for the methylated dcm site on GUS DNA) compared to that of the non-treated plasmid, indicated that demethylation had occurred. DNA demethylation by 5-azaC appears to be one explanation for the observed increased GUS

expression. Proposed mechanisms of demethylation will be presented and discussed.

- P-1044** Somatic Hybridization Of *Sinapis alba* And Rapid Cycling *Brassica oleracea*: A Step Toward Transfer Of Pest Resistance Into *Brassica* Vegetables. L.N. HANSEN and E.D. Earle. Department of Plant Breeding, Cornell University, Ithaca, NY 14853.

*Alternaria* spp., causing black leaf spot, and the cabbage maggot are both serious pests of cruciferous crops, including all *Brassica oleracea* vegetables. Sufficient resistance is not available in this species, nor in species that readily cross sexually with *B. oleracea*. *Sinapis alba* or white mustard contains good resistance to both pests. Since crossing the two species sexually is very difficult, an alternate way of gene transfer must be used. This is offered by protoplast fusion, which may overcome the intergeneric crossing barrier. Somatic hybrids have been produced by protoplast fusion between a rapid cycling line of *B. oleracea* with good regenerability and a *Sinapis alba* line selected for high resistance to *Alternaria* and good performance in protoplast culture. All 25 regenerated plants have a morphology intermediate to the two parental species. Flow cytometric analysis showed nuclear DNA contents similar to the sum of the two parents. Hybrid identity was further confirmed by LAP and PGI isozyme analysis. The plants are currently being propagated by cuttings to produce material for screening for resistance to *Alternaria* as well as to the cabbage maggot.

- P-1045** A Simple, Reliable Transformation Method For *Brassica oleracea*. T.D. METZ, R. Dixit, and E.D. Earle. Department of Plant Breeding, Cornell University, Ithaca, NY 14853.

*Brassica oleracea* consists of several important vegetable crops including broccoli, cauliflower, kale, and cabbage. The genetic transformation of some *Brassica oleracea* varieties has proven to be quite difficult. While several accounts of successful transformation of *Brassica oleracea* have been published, most procedures have limitations which prevent them from being widely applicable. We have successfully produced transgenic broccoli which express a *Bacillus thuringiensis* insect control protein using a modification of a recently published *Agrobacterium tumefaciens*-mediated method. While we have had repeated success using this system, which utilizes flowering stalk segments as the source of explants, there are several disadvantages with using full grown flowering plants. Experiments are difficult to schedule and require 4-6 months of advance planning, greenhouse space and plant maintenance is necessary, and frequent fungal and bacterial contamination of the explants after surface sterilization often require the experiments to be repeated, causing costly time delays. We report an efficient *Agrobacterium tumefaciens* transformation method for *Brassica oleracea* which utilizes 14-21 day old *in vitro* grown seedling explants. We have transformed broccoli, cabbage and rapid cycling *Bras-*

*sica oleracea* lines using this method and are able to regenerate rooted transformants routinely in 3 months. The results of this method, including explant regenerability, ploidy levels of regenerants, and transformation efficiency are compared to those of the flowing stalk transformation method.

**P-1046** The Effects Of Seedling Age And Light Intensity On *Brassica napus* L. Transformation And Regeneration Frequencies. C. LI and J.C. Turner. Calgene Inc., 1920 Fifth Street, Davis, CA 95616.

*Brassica napus* L. (rapeseed) has been widely used as a host for *Agrobacterium*-mediated genetic transformation. When hypocotyl explants are used for cocultivation, seedling germination conditions may influence cell susceptibility to *Agrobacterium* infection and subsequent regeneration. The effect of germination conditions on transformation and shoot regeneration is currently under investigation. Variables being studied include seedling age, light intensity, and use of a dark period prior to the cutting of explants. Transformation and shoot regeneration frequencies will be reported.

**P-1047** Regeneration And Transformation Of *Arachis* Spp. M. Cheng<sup>1</sup>, Z. Li<sup>1</sup>, A. Xing<sup>1</sup>, R.L. Jarret<sup>2</sup>, R.N. Pittman<sup>2</sup>, and J.W. Demski<sup>1</sup>. <sup>1</sup>Department of Plant Pathology, Georgia Station, University of Georgia, Griffin, GA 30223; <sup>2</sup>USDA-ARS Regional Plant Introduction Station, Georgia Station, 1109 Experiment Street, Griffin, GA 30223.

Somatic embryogenesis was induced from cultured embryo axes, and embryonated cotyledon explants isolated from dry seeds, of 5 *Arachis hypogaea* cvs. and *A. monticola*. Explants were cultured on MS media with various plant growth regulator treatments. Significant differences among genotypes and medium treatments were observed. Runner and Virginia types were more responsive than Valencia or Spanish types. Somatic embryogenesis was induced in 96% of cv. Florunner explants cultured on MS with 3 mg/L picloram. Somatic embryogenesis also occurred when embryo axes from dry seed and epicotyl sections from seedling of *A. monticola* were cultured on this media. Epicotyl and petiole sections obtained from in vitro cultured seedlings and from greenhouse-grown plants of *A. villosulicarpa* were incubated on MS medium supplemented with various combinations of plant growth regulators including: 6-benzyladenine (BA), naphthaleneacetic acid (NAA), BA, 2,4-dichlorophenoxyacetic acid (2,4-D), picloram (PIC), thidiazuron (TDZ), indole-3-acetic acid (IAA) and kinetin. Shoot primordia were regenerated from epicotyl explants cultured on MS with 1 mg/L TDZ + 1 mg/L IAA. Fifty percent of these bud primordia developed into normal shoots after transfer to MS medium supplemented with 0.5 mg/L BA + 0.5 mg/L GA and 1 mg/L AgNO<sub>3</sub>. Cell cultures were established by inoculating the calli initiated on MS medium with 1 mg/L 2,4-D + 1 mg/L BA in liquid MS medium containing 2 mg/L BA + 2 mg/L NAA. When this callus was transferred directly to MS

medium supplemented with 1 mg/L BA + 1 mg/L NAA, shoot formation occurred. Five constructs including 35S:peanut chorotic streak virus coat protein gene (pcsv-cp), double 35S:pcsv-cp, double 35S TMV:pcsv-cp, double 35S TEV:pcsv-cp and pcsv promoter:pcsv-cp have been constructed in a binary vector. All the constructs have been introduced into *Agrobacterium* EHA 105 by triparental mating. These regeneration systems are currently being used for *Agrobacterium*-mediated transformation.

**P-1048** Co-Transformation And Subsequent Segregation Frequencies In Tobacco Utilizing An *Agrobacterium* Strain Containing Two Binary Plasmids. M. DALEY and V. Knauf. Calgene, Inc., Davis, CA 95616.

Co-transformation and subsequent segregation frequencies were investigated in tobacco using *Agrobacterium tumefaciens* strain harboring two binary plasmids. One of the binary plasmids contained a selectable marker gene, while the other contained a reporter gene. Plants expressing both genes were identified and self-pollinated. Seeds from these co-transformed plants were germinated, and the seedlings were analyzed for the presence of the marker and reporter genes. Co-transformation and co-segregation frequencies obtained demonstrate the possibility of using co-transformation and subsequent segregation to eliminate a selectable marker gene from plant lines in which a gene of interest and a marker gene were inserted on different chromosomes.

**P-1049** Effect Of Overexpression Of SOD On Growth Of Transgenic Plants Under Drought And Low Temperature. N.L. TROLINDER and R.D. Allen. USDA-ARS, CSRL, Lubbock, TX 79401 and Texas Tech University, Lubbock, TX 79409.

Transgenic plants that over-express superoxide dismutase (SOD) have been developed in several laboratories. Although there are exceptions, these plants have been shown to have increased protection from oxidative stress caused directly by treatment with paraquat or indirectly through stress exposure. We have begun to test whether this approach is applicable for increasing stress tolerance in cotton. We used a chimeric gene developed by Dr. C. Bowler that contains coding sequences for the mature Mn SOD subunit from *Nicotiana glauca* fused with a chloroplast transit peptide sequence from an *Arabidopsis thaliana* RUBISCO gene. This coding sequence is expressed under the control of a CaMV 35S promoter. The chimeric Chl-Mn SOD gene construct was transferred to cotton plants via an *Agrobacterium*-mediated transformation procedure. Regenerated transgenic cotton plants were analyzed for the expression of novel SOD isoforms using a non-denaturing polyacrylamide gel negative staining technique. A unique SOD isoform was identified in extracts of several transgenic SOD cotton plants. Tests of the oxidative stress resistance of transgenic cotton plants that express chloroplastic Mn SOD are currently under way. These include characterization of their paraquat

resistance, analysis of photosynthesis during and after exposure to high light intensity and low temperature and drought tolerance.

- P-1050** Development Of Marker System In Wild *Nicotiana* Species Via Direct Gene Transformation. V. Ilcheva<sup>1</sup> and M. Vlahova<sup>2</sup>. <sup>1</sup>Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia; <sup>2</sup>Institute of Genetic Engineering, 2232 Kostinbrod-2, Bulgaria.

Somatic hybridization is an approach to overcome the incompatibility barriers in interspecific hybrids. An appropriate marker system of one or both partners is needed to achieve selection of heterokaryons after protoplast fusion. Transgenic kanamycin resistant calli were obtained in wild *N. rotundifolia* and *N. africana*. Transformation was performed using 10 mg/ml pHB2 plasmid carrying npt II gene and 35S promoter alone or with an addition of 50 mg/ml salmon sperm carrier DNA and 30% PEG 4000 for 12 min. The results showed that protoplast incubation with plasmid DNA combined with carrier DNA increases the transformation rate. It was twice higher ( $2.7 \times 10^{-5}$ ) in *N. rotundifolia* than the experiment with plasmid DNA alone ( $1.3 \times 10^{-5}$ ). Transgenic calli in *N. africana* were obtained only when carrier DNA was applied ( $0.14 \times 10^{-5}$ ). The transformants could be a suitable donor material in further somatic hybridization experiments.

- P-1051** A Glyphosate-tolerant EPSP Synthase Gene As A Selectable Marker In Wheat Transformation. H. ZHOU, J.E. Fry, J.W. Arrowsmith, T.W. Corbin, and M.E. Fromm. Monsanto Company, Mail Zone GG4H, 700 Chesterfield Parkway North, St. Louis, MO 63198.

Transgenic wheat plants tolerant to bialaphos have been previously obtained through particle gun bombardment of immature embryo calli. We have developed an efficient transformation system and have demonstrated this system using a glyphosate-tolerant EPSP synthase gene as a selectable marker. A large number of transgenic wheat plants have been produced. Southern blot, ELISA, and Western blot analyses confirmed the integration and expression of the EPSP synthase gene in the transgenic plants. The transgene is transmitted to the following generations in a Mendelian fashion.

- P-1052** Bulblet Formation From Three Varieties Of *Allium sativum* In Vitro. M.L. CARDENAS<sup>1</sup>, T.E. Torres<sup>1</sup>, R. Mercado<sup>1</sup>, J.A. Villarreal<sup>2</sup>, and E. Cárdenas<sup>3</sup>. <sup>1</sup>UANL A.P. 2790 San Nicolás Gza. N.L.; <sup>2</sup>U. R. Mty; <sup>3</sup>Facultad de Agronomía UANL. Apdo. 358 San Nicolás Gza. N.L. Mexico.

Garlic plants produced through tissue culture are useful for improving yield and quality of the crop. Three commercial varieties of garlic (*Allium sativum*) used in Mexico (Cadereyta, Celaya, and Taiwan) were tested on this investigation to produce maximum growth and bulblet formation from shoot tip culture. Cloves were surface disinfected by soaking in 70% ethanol for 30 seconds, then in 1.2% sodium hypochlorite solution for 15 min and

then rinsed three times in sterile water. The cloves were maintained at 5° C for two weeks in 0.8% agar. Shoot tips with two primordia (3-5 mm in height), were excised aseptically from cloves and cultured for 2 months on Murashige-Skoog or Gamborg, Miller and Ojima media, containing several combinations of plant growth regulators (0.01, 0.03 IAA and 0.1, 1.0 Kinetin). In general, the maximum rate of growth was achieved with the highest levels of IAA and Kinetin. Several replications were maintained for five months to observe in vitro bulblet formation.

- P-1053** Micropropagation From Shoot Tips Of Tomato (*Lycopersicon esculentum* [Mill]). J. AMBRIZ, E. Olivares, E. Cárdenas, and F. Montes. Facultad de Agronomía UANL Apdo. Postal 358, San Nicolás de los Garza, N.L. 66420, Mexico.

Although in the micropropagation of herbaceous species, the enhanced axillary and terminal buds result less efficient, because the number of plants obtained is determined for number of preexistent buds in explant; advantage of this technique, unlike other morphogenic processes is that the regenerants show a high genetic stability. The study involved the proliferation *in vitro* of two tomato hybrids: Celebrity and Heat Wave. It used epicotyls of 5 mm in length from seedlings germinated in agar 0.8%. Shoot proliferation was achieved in MS medium supplemented with 3% sucrose, 2 mg·l<sup>-1</sup> glycine, 0.5 mg·l<sup>-1</sup> pyridoxine.HCl, 0.5 mg·l<sup>-1</sup> nicotinic acid, 0.1 mg·l<sup>-1</sup> thiamine, 100 mg·l<sup>-1</sup> myo-inositol, 0.5% agar-gele and 1 mg·l<sup>-1</sup> of IAA. Same results were obtained combining with BAP, kinetin or zeatin. The *in vitro* root stage was possible when the explant was transferred to the same medium but without growth regulators or by adding 1 mg·l<sup>-1</sup> of IBA. The survival of the regenerated plants was 100%.

- P-1054** Automated Sucrose Concentration Control In Micropropagation Media. S.A. Hale and R.E. Young. Biological and Agricultural Engineering Department, North Carolina State University, Raleigh, NC 27695-7625; and Agricultural and Biological Engineering Department, Clemson University, Clemson, SC 29634-0357.

A whole plant liquid/membrane micropropagation bioreactor system has been modified to allow the culture process to operate semi-continuously. The media composition control (MCC) system allowed bioreactor media to be automatically sampled and an aliquot analyzed for sucrose and dextrose concentration determination. Data obtained were used as an input to an automatic control system which determined culture chamber media volume and maintained both media sucrose concentration and volume levels within  $\pm 5$  percent and  $\pm 2.5$  percent tolerances, respectively. MCC control system performance testing, without explant tissue, showed it to respond satisfactorily to disturbances and set point changes. Separate culture performance testing conducted with stage 1 *Nicotiana tabacum* (tobacco) explant cultures compared tissues growth over a 28-day

period in the MCC system with that of tissues grown in a liquid/membrane bioreactor with 7-day media replenishment and tissues grown in a MCC-like system which only monitored sucrose concentration. Average fresh weight gains for MCC cultured tissues were found to be 1.5 times larger than liquid/membrane bioreactor grown explants and 7.8 times greater than explants in the MCC-like, sucrose monitoring system.

**P-1055** In Vitro Clonal Propagation Of Country Potato—An Under-Exploited Tuber Plant. J. Ponsamuel<sup>1</sup>, N.P. Samson and K. Paul Anderson<sup>2</sup>. Plant Tissue Culture Unit, Madras Christian College, Madras 600 059, India. <sup>1</sup>Current Address: Plant Biology Division, The Samuel Robert's Noble Foundation, P.O. Box 2180, Ardmore, OK 73402; <sup>2</sup>National Plant Tissue Culture Repository, NBPGR, New Delhi 110012, India.

Country potato (*Coleus parviflorus* Benth cv. CP-9) is an under-exploited tuber crop and is cultivated in a limited scale in many parts of India. Its nutritive quality is comparable to the common potato, and it has the potential of developing into a crop similar to potato. A protocol for the in vitro regeneration of country potato was developed in our lab. The explants used were shoot tips, leaf, nodal (NO), and internodal (IN) segments isolated from 10-day-old auxenic sprouts. The shoot tip and the NO explants developed multiple shoots within 20 d of primary culture in 10  $\mu$ M 2-isopentynyladenine (2-iP) or 10  $\mu$ M Benzylaminopurine (BAP) supplemented MS gel medium. Leaf explants on 10  $\mu$ M 2-iP + 1  $\mu$ M Tetraphenylboron (TPB) developed direct shoots 30 d after culture initiation. This treatment produced the maximum number of shoots, when subcultured in the same media. Such direct caulogenesis was also observed in 10  $\mu$ M BAP + 1  $\mu$ M -Naphthaleneacetic acid (NAA) and also in 10  $\mu$ M BAP + 1  $\mu$ M Phenylboronic acid (PBOA) treatments. PBOA + BAP combination induced 22% more shoots than the NAA + BAP combination. The NO and IN explants induced callus 7 d after culture initiation. After 25 d, the cut surface of the explants differentiated ~1 cm<sup>3</sup> green and compact callus. The combination treatment, 10  $\mu$ M BAP + 1  $\mu$ M TPB induced the maximum percentage of this compact callus. The compact callus produced shoots 15 d after the subculture on MS medium with 10  $\mu$ M 2-iP or 10  $\mu$ M BAP. The shoots thus developed from all the explants were dismembered and subcultured onto auxin free MS medium for root induction. After 10 d, the rooted plants were acclimatized in mist chambers by transferring them to presterilized Soilrite with 1/2 MS and 10  $\mu$ M Indole-3-butyric acid. The plant recovery rate was 90%, and after 17 d the established plants were transferred to the soil for field testing.

**P-1056** Cotton Fiber As A Substitute For Agar Used In Plant Tissue Culture. R.M. CERDEIRA<sup>1</sup>, J.V. Krans<sup>2</sup>, J.D. McChesney<sup>1</sup>, A.M.S. Pereira<sup>3</sup>, and S.C. Franca<sup>3</sup>. <sup>1</sup>Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS 38677; <sup>2</sup>Department of Agronomy, Mississippi State University, MS 39762. <sup>3</sup>UNAERP, Ribeirao Preto, SP 14100, Brazil.

Cotton fibers were tested as a substitute for agar in plant tissue culture studies. The high cost of agar has contributed to a search for an alternative medium support. Medium support was evaluated in terms of callus maintenance, plantlet regeneration, and micropropagation using different species: *Artemisia annua* L., *Agrostis stolonifera* cv. *palustris* Farwell, *Taxus x media* cv. *Hicksii* Rehd, and *Cephaelis ipecacuanha* Rich. *Taxus* and *Agrostis* calli cultivated on liquid media with cotton fiber as media support (4% w/v) showed higher growth than calli on agar (0.8% w/v). There was no significant difference between *Agrostis* plantlet differentiation using calli cultivated on cotton support medium or agar support. For *Artemisia* and *Agrostis* micropropagation, the number of microshoots induced on media supported with cotton was not different than the number of microshoots cultured on agar.

**P-1057** The Effect Of BA And NAA In *Podophyllum* Shoot And Rhizome Culture. R.M. CERDEIRA, C. Burandt, Jr., and J.D. McChesney. Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS 38677.

Shoot and rhizome cultures have been established from rhizome explants of *Podophyllum peltatum* L. After 45 days of cultivation on MS salts with different ratios of BA and NAA, shoots and rhizomes were induced. Rhizome growth was maintained on MS half strength with 1.0 mg/L of kinetin and 1% PVP. These cultures will be evaluated for their ability to produce podophyllum toxin.

**P-1058** Factors Affecting High Frequency Shoot Regeneration From Leaf Explants Of Muskmelon; Enhancement By The Sulfonylurea Herbicide, Pinnacle. R.C. YADAV<sup>1</sup>, M. Saleh<sup>2</sup>, and R. Grumet<sup>1</sup>. <sup>1</sup>Michigan State University, East Lansing, MI 48824; <sup>2</sup>Agricultural Genetic Engineering Research Institute, Giza, Egypt.

An efficient plant regeneration system is a prerequisite for successful transformation experiments. We studied the effect of different growth conditions of the source plants and different media supplements on regeneration from melon leaves. Shoots were successfully regenerated from leaf explants cultured on melon regeneration (MR) medium (Niedz et al., 1989) at 25  $\pm$  2° C under a 16 h light/8 h dark photoperiod. Media components such as acetone, silver nitrate, and phytigel (vs. bactoagar) were tested. Silver nitrate and phytigel enhanced regeneration. Growth conditions prior to explant preparation were critical. Leaf explants from greenhouse and growth chamber grown plants gave a high percent regeneration (up to 90 and 60%, respectively), but there was no regeneration from plants grown in Magenta

boxes in the culture room. Leaf age was also important; explants from small leaves (3 cm diameter) performed better than those from older leaves. Unexpectedly, when tests were performed to establish screening levels for transgenic plants for resistance to the sulfonylurea herbicide, Pinnacle (an inhibitor of branched chain amino acid biosynthesis), there was a dramatic enhancement of regeneration. Low levels of the herbicide gave up to 3-fold increase in shoot regeneration.

**P-1059** Behavior Of Rhododendron Tissue Affected By Tissue Proliferation. N.H. BRAND and R. Kiyomoto. Department of Plant Science, University of Connecticut, Storrs, CT 06269 and Connecticut Agricultural Experiment Station, New Haven, CT 06504.

Tissue proliferation (TP) is a recently-observed malady affecting micropropagated rhododendron, where plants produce basal callus/tumors, often accompanied by excessive adventitious shoot production. Because little is known about TP, some basic studies were initiated to characterize in vitro growth of tissues from plants exhibiting tissue proliferation (TP+) and from plants free of tissue proliferation (TP-). Shoot tip cultures were initiated from TP+ and TP- plants of *Rhododendron* 'Montego' on Woody Plant (WP) medium containing 10  $\mu\text{M}$  2-isopentenyladenine (2iP). Shoot tips from TP- plants required 10-12 months to miniaturize and initiate multiplication, while shoot tips from TP+ plants required only 4 months. TP+ cultures could not be maintained on 10  $\mu\text{M}$  2iP medium beyond 6 months due to excessive proliferation of short, abnormal shoots and callusing. Once initiated, TP+ shoot cultures could be maintained on hormone-free medium where they continue to grow and multiply. This "habituated" state has been maintained for 8 months to date. Nodal and internodal tumors, altered leaf and stem morphology and free axillary branching distinguish TP+ cultures from TP- cultures. Morphological aberrations have been stable for 5 years outside of the culture environment, even following subsequent repropagation via shoot cuttings. To study callus growth, calli from TP+ and TP- *Rhododendron* 'Catawbiense Album' stem segments were placed on WP medium containing auxin and 2iP. After the first subculture period, TP+ calli grew significantly faster than TP- calli.

**P-1060** Micropropagation Of A Dwarf Trumpet Vine, *Campsis* sp. W.A. MACKAY. Texas A&M University Research and Extension Center, 1380 A&M Circle, El Paso, TX 79927.

Actively growing shoots were collected from mature dwarf trumpet vine in central Texas and successfully cultured on basal medium consisting of MS salts and vitamins, 30 g $\cdot$ l $^{-1}$  sucrose, 0.8% Phytagar supplemented with 1.0 mg $\cdot$ l $^{-1}$  BA. Experiments were conducted to optimize shoot proliferation. Factors examined included salt formulations and concentrations, growth regulators, sucrose concentrations, and gelling agent materials. Shoots readily rooted when transferred to basal medium containing NAA 0.1 mg $\cdot$ l $^{-1}$ . Of 150 plants established in

the greenhouse, only two exhibited the parental dwarf phenotype. The remaining plants were vigorous vine type plants with altered leaf morphology. All of the regenerants exhibited flower morphology similar to the parental material. However, the vine type regenerants exhibited a greater number of nodes to first flower than the dwarf phenotype. Several hypothesis for this phenomenon will be discussed.

**P-1061** Influence Of Thidiazuron, Gibberellic Acid, And Abscisic Acid On *In Vitro* Shoot Formation From Excised Roots Of Silk tree. D. Sankhla, TIM D. DAVIS, and N. Sankhla. Texas A&M University Research and Extension Center, 17360 Coit Road, Dallas, TX 75252-6599.

Silk tree (*Albizia julibrissin*) is a leguminous ornamental tree grown in U.S. landscapes. For our gene transfer work, we were interested in developing an efficient regeneration protocol for this plant. This report describes the response of excised roots to thidiazuron (TDZ), gibberellic acid (GA<sub>3</sub>), and abscisic acid (ABA) in the culture medium. Without growth regulators, about 50% of the excised roots placed on Gamborg's B5 medium formed shoot buds within 15-20 days. Addition of low levels of TDZ (.01-0.1  $\mu\text{M}$ ) to the medium increased the percentage of cultures which formed shoots (to 90-95%) and the number of shoots formed per culture. At higher TDZ levels ( $\geq 1$   $\mu\text{M}$ ), intense callusing occurred but organogenesis was inhibited. Both GA<sub>3</sub> (0.3-3  $\mu\text{M}$ ) and ABA (.04-0.4  $\mu\text{M}$ ) were potent inhibitors of shoot formation. In fact, most explants reared on ABA did not proliferate and soon became black. A combination of TDZ with GA<sub>3</sub> or ABA abolished the inhibitory action of the latter chemicals on shoot formation. In the medium containing the lowest level of ABA with TDZ, short and sturdy shoots were produced along the entire length of the explant. In the medium containing both TDZ and GA<sub>3</sub>, shoots that formed were elongated with pale-green leaves. Upon excision from the original explant, the regenerated shoots readily formed adventitious roots when subcultured onto fresh B5 medium. Thus, with an appropriate combination of growth regulators, high frequency regeneration of shoots from excised roots can be obtained.

**P-1062** Micropropagation Of *Indica* Rice Through Proliferation Of Axillary Shoots. J.S. SANDHU, S.S. Gosal, M.S. Gill, and H.S. Dhaliwal. Downing College, University of Cambridge, CB2 1DQ, U.K.; Plant Biotechnology Centre, Punjab Agricultural University, Ludhiana, 141004, India.

Week old seedlings of *indica* rice variety Jaya obtained on basal MS medium and further subcultured on agar solidified MS medium supplemented with cytokinins, sucrose (3% w/v) and mannitol (1% w/v) lead to development of multiple shoot buds. Shoot cultures were maintained and multiplied in liquid medium containing BAP 5 mg l $^{-1}$ , sucrose (3% w/v) and mannitol (1% w/v). Profuse rooting was obtained on transfer to MS liquid medium containing IBA 1 mg l $^{-1}$  and sucrose (3% w/v).

Complete plants were successfully transferred to soil and grown to maturity.

- P-1063** Somaclonal Variation In Tissue Culture Derived Plants Of Pigeonpea. CH. PRASANALATHA<sup>1,2</sup>, J.P. Moss, K.K. Sharma<sup>1</sup>, and J.K. Bhalla<sup>2</sup>. <sup>1</sup>Cellular and Molecular Biology Division, ICRISAT, Patancheru 502324, A.P., India; <sup>2</sup>Cytogenetics and Tissue Culture Lab., Department of Botany, Osmania University, Hyderabad 500 007, India.

Somaclonal variation in crop plants is a consequence of plant regeneration from in vitro culture, and is now considered to be a potential source of genetic variability which can be used in plant improvement programs. Thus, tissue culture methods leading to somaclonal variation can be used to introduce agronomically important traits like disease and insect resistance, salt and drought tolerance, which are not readily available in the pigeonpea germplasm. Methods were developed to obtain high frequency regeneration of adventitious shoot buds from cotyledons explanted from 7-day-old seedlings. These shoot buds were elongated, rooted and transplanted to the glasshouse. Some of the regenerated plants ( $R_1$  generation) exhibited a continuous spectrum of floral variations such as petaloid sepals, 2 standard petals, 2-4 wing petals, split keel, 12-14 anthers, and 2-3 gynoecea (which formed twin or triple pods). Some flower buds had anthers which protruded even before the flower opening. The preliminary results from the  $R_2$  generation plants that were planted under field conditions revealed segregation with respect to flower color, plant height, leaf shape, and growth habit (indeterminate and semi-determinate types and erect and compact types). In this population one plant completely failed to set pod in spite of profuse flowering. This occurrence of phenotypic variation in the  $R_1$  plants and their progeny is an indication of somaclonal variation in the tissue culture system of pigeonpea.

- P-1064** Micropropagation Of Pomegranate Jyoti From Axillary Bud Explants. A. MURALIKRISHNA and A.N.S. GOWDA\*. H.No. 171, "Sreshta" I Phase, Maharani Avenue, Vadavalli (P.O.) Coimbatore 641 041, Tamil Nadu, India. \*Division of Horticulture, University of Agril, Sciences, GKVK, Bangalore, India 560 065.

Suitable micropropagation strategies were developed for commercially popular cultivar of Pomegranate (*Punica granatum* L.) cv. Jyoti. Inherent difficulties associated with exudation and oxidation of polyphenols and/or tannins and consequent browning of explants in the culture media was alleviated by using half strength Murashige and Skoog's basal (MSB) medium combined with frequent transfers initially up to six times per week. The maximum number (8.43) of adventitious/multiple shoots were produced from axillary bud explants on MSB with 6-Benzyl amino purine and Kinetin each at 0.50  $\mu$ M concentration. They were further subcultured at every 42 days for six or more times until they were transferred to rooting media. These regenerated shootlets, pre-soaked in Indole-3-butyric acid at 100 mg/l

<sup>1</sup> for one hour and transferred onto MSB free of plant growth regulators resulted in better rooting (40%) compared to addition of auxins in the media. Among the different potting media tried vermiculite were most suitable media for growth of pomegranate plantlets in pots.

- P-1065** The Effects Of Methionine Sulfoximine On Protein And RNA Synthesis Of Soybean. \*E.L. Myles, M. Zheng, and C. Caudle. Tennessee State University, Nashville, TN 37209.

The bacterium, *Pseudomonas syringae* pv. *glycinea*, causes the disease Bacteria Blight in soybean. It produces a pathogenic toxin (tabtoxin) that inhibits glutamine synthetase activity, thus causing cell death in plants. Methionine Sulfoximine (MSO) is commercially available and therefore an adequate substitute for studying the effects of bacterial blight disease in soybean. In this study, 0.5 grams of callus was placed on media containing 0, 2, 4, 8, 12, and 16 mM MSO. After 4 weeks, the weights were recorded. The callus was frozen with liquid nitrogen and proteins or total RNA was extracted. The proteins were analyzed by one- and two-dimensional polyacrylamide gel electrophoresis and by using a BIO-RAD video densitometer. The total RNA was separated by size in a formaldehyde denaturing 1% agarose gel. Results showed that the total weight of callus tissue from Pioneer hypocotyls increased. The weight of stems and roots from Weber and Pioneer decreased with each increment of MSO. The average total amount of protein per gram tissue decreased in response to MSO in Weber and Pioneer hypocotyls, but increased in Pioneer root and stem. The total amount of RNA increased in 4 and 8 mM MSO in Weber. RNA synthesis may be directly involved in resistant reaction of soybean to bacterial blight. Densitometer analysis demonstrated that resistant expression may require *de novo* synthesis of certain new proteins.

- P-1066** The Effects Of Atrazine On Protein Synthesis In Soybean Callus. P.S. KAHN and S.M. Bhatti. Cooperative Agricultural Research Program, Tennessee State University, Nashville, TN 37209-1561.

Residual atrazine causes soybean injury when soybean is rotated with corn. The development of soybean cultivars which are genetically tolerant to atrazine is therefore of great importance. Our objectives were 1) to compare proteins in adapted and non-adapted cells in order to identify the proteins which may play a role in offering resistance and 2) to determine whether embryogenic tissue was more or less sensitive to atrazine than undifferentiated tissue. The leaf explants of soybean cultivars 'Weber,' 'Pella' and 'Forrest' were exposed to various levels of atrazine (0, 10, 15, 25, and 50  $\mu$ M) added to  $L_6$  solid medium. The results showed that at 50  $\mu$ M atrazine, the frequency of embryo formation dropped by 50% as compared to control. At 10, 15, and 25  $\mu$ M, there was no significant variation from control. Quantitative analysis of total protein showed that at atrazine levels as low as 25  $\mu$ M, there was a 50% reduction in total protein. At 50  $\mu$ M atrazine, the total protein was only 35%

of control. The sensitivity of embryogenic callus to atrazine remained the same after subsequent subculture. (Supported by USDA/CSRS Project #TENX-9403-14-PS34).

**P-1067** Genotype Specific Polypeptide Induction With Abscisic Acid And Water Stress In Callus Cultures Of Rice. K. UMA RANI and G.M. REDDY. CPMB, Osmania University, Hyderabad 500 007, India.

Polypeptide changes induced by water stress and ABA treatment were studied with N22, a drought tolerant genotype and Tellahamsa (TH), a susceptible genotype. Embryo derived calli (30 day old) was subjected to 5% (w/v) Polyethylene glycol and 20  $\mu$ M Abscisic acid (ABA), the proteins were resolved on two-dimensional polyacrylamide gels. In the tolerant genotype N22, seven polypeptides were induced compared to two polypeptides in susceptible genotype TH by ABA and water stress, besides this, ABA induced two additional polypeptides of 16.5 kD and 21 kD in TH as revealed by one-dimensional (SDS-PAGE). Two-dimensional analysis revealed 10 polypeptides with ABA and water stress in N22, which were of low mol. wt., 14.12 to 26 kD with an isoelectric point (pI) ranging from 5.50 to 8.45. However, in TH, ABA and water stress exhibited differential protein changes, eight polypeptides were induced by ABA and water stress with mol. wt. and pI in the range of 24 to 14.7 kD, and 5.0 to 9.2, respectively. ABA specifically induced 21 kD polypeptide with pI of 6.75. Water stress and ABA induced similar responses in tolerant genotype N22 and differential responses in susceptible genotype TH, suggesting the differences in "responsive" elements regulating the synthesis of these polypeptides with ABA and water stress in N22 and TH. The transcriptional and/or post-translational modification(s) may be responsible for synthesis of proteins by ABA during water stress.

**P-1068** Effects Of Cold Preincubation, Aminoxyacetic Acid And Cobalt On Ethylene Emanation And Somatic Embryogenesis From Orchardgrass Leaf Cultures. A.I. KUKLIN, Z. Tomaszewski, Jr., C.E. Sams, and B.V. Conger. Plant & Soil Science Department, University of Tennessee, Knoxville, TN 37901.

The objective of this investigation was to study the relationship between ethylene emanation and somatic embryogenesis from orchardgrass (*Dactylis glomerata* L.) leaf cultures after treatment with low temperature, aminoxyacetic acid and cobalt. Low temperature (4°C) preincubation of segments from the innermost leaves cultured on SH medium with 30  $\mu$ M dicamba for 1 to 7 d before transfer to 21°C significantly decreased ethylene emanation. Results from a paired design showed that the embryogenic response of leaf segments preincubated at 4°C was equal or superior to nonpreincubated leaves at all time periods. Aminoxyacetic acid at 20 and 40  $\mu$ M decreased ethylene emanation but did not stimulate the embryogenic response. Cobalt concentrations greater than the basal level (0.42  $\mu$ M) in SH medium significantly decreased ethylene emanation without changing the number of somatic embryos. We conclude that the

stimulation of somatic embryogenesis by low temperature is probably due to factors other than suppression of ethylene biosynthesis. An incomplete block design in combination with a hierarchical design was used to determine possible sources of variability in the somatic embryogenesis response.

**P-1069** In Vitro Selection For Salt Tolerance In Cultivars Of Indica Rice. J. Ponsamuel\* and Elmar W. Weiler. Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität, Postfach 102148, 44803 Bochum, Germany. \*Current Address: Plant Biology Division, The Samuel Robert's Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Callus was induced from seed explants of indica rice cultivars IR-50, IR-60, J-13, JAYA, and PONNI on MS gel medium (0.8% Agar) supplemented with MS vitamins 20 g/l sucrose, 10  $\mu$ M 2,4-Dichlorophenoxyacetic acid. The cultures were incubated in darkness at 28°C. For selection, the calli were transferred to liquid MS medium of similar composition containing 0.5% NaCl. During subsequent subcultures (30 day intervals), the NaCl concentration was gradually increased to 2.5% at the rate of 0.5% each time. Cell cultures tolerant to salt concentration up to 2% were obtained only from the cultivars IR-60 and J-13, and subsequent transfer of the survived cell clumps to 2.5% salt induced senescence and cell necrosis. Conversely, the cell clumps of cultivars IR-50, JAYA, and PONNI succumbed at salt concentration as low as 0.5%. The selected cell clumps of J-13 cultivar from 2% salt produced shoots on MS gel medium with 3  $\mu$ M Benzylaminopurine + 0.2  $\mu$ M of  $\alpha$ -Naphthaleneacetic acid and a photon density of 0.45  $\mu$ E/m<sup>2</sup> s<sup>-1</sup> from cool fluorescent white light. These shoots rooted on hormone and NaCl free MS gel medium. Although IR-60 cell suspension survived 2% salt, it remained nonembryogenic in the regeneration medium. Regenerated plants (R<sub>0</sub>) of J-13 were transferred to soil and grown to maturity in the greenhouse. The seeds collected from R<sub>0</sub> plants germinated in 2% NaCl. The plants raised from these seeds were tolerant to the same concentration of salt.

**P-1070** Influence Of Cd and Pb On Growth, Proline Level In Calli Of Two Species *Nicotiana*. P. Nikolova<sup>1</sup>, J. Topalova<sup>2</sup>, S. Chankova<sup>3</sup>, G. Nickoff<sup>4</sup>. <sup>1</sup>Higher Inst. of Chem. Techn., Sofia, Bulgaria 1156; <sup>2</sup>Faculty of Biology, Sofia University, Sofia, Bulgaria 1421; <sup>3</sup>Institute of Genetics, BAS, Sofia 1113; <sup>4</sup>Central Lab. of Biophysics, BAS, Sofia, Bulgaria 1113.

Stem segments from *Nicotiana tabacum* (cv. Ludogorets) and *N. alata* were cultivated in Murashige and Skoog agar medium with supplements for callus induction. After 20 days, parts of the well-developed calli were removed into fresh medium used as control and the rest remained in medium with 0.02, 0.04, 0.06, 0.13 M CdCl<sub>2</sub> and 0.9, 1.8, 3.6 M PbCl<sub>2</sub>. Their weight was measured before and after 30 days treatment. The influence of Cd and Pb is characterized according to the relative growth rate of callus and accumulation of proline.

An inhibition of fresh weight of the callus was established. Wild species of *N. alata* expresses small level of inhibition. Progressive proline accumulation with the increase of Cd and Pb concentration is observed. Besides, the results obtained show that Cd in concentration 0.02 M induced organogenesis. The values of proline content for the respective Cd and Pb concentration at *N. alata* are higher in comparison with *N. tabacum*. The presented results confirm the higher resistance of the wild type towards Cd and Pb and the role of proline content as biological indicator for stress influences.

**P-1071** Carbon And Nitrogen Utilization By Soybean Seeds Grown In Vitro. C.H. Saravitz and C.D. Raper, Jr. Department of Soil Science and Plant Physiology Program, Box 7619, North Carolina State University, Raleigh, NC 27695-7619.

Embryo culture was used to examine how changes in the availability of carbon and nitrogen from the mother plant affect both accumulation of dry matter and embryo composition. In vitro maturation of soybean seeds provides an environment to study the effects of known concentrations of nitrogen and carbohydrate on seed development and determine whether fluxes of either carbon or nitrogen into the seed change as availability of the other varies.

Immature soybean embryos were aseptically excised 17 days after flowering and grown in modified MS medium containing different concentrations of sucrose and glutamine. Embryos were transferred to fresh medium every 4 days to maintain sucrose and glutamine concentrations of the culture medium. In all experiments, accumulation of dry matter and nitrogen content were greatest when the sucrose concentration of the culture medium was 150 mM, decreased with lower sucrose concentrations (1.5 or 15 mM), and either remained constant or decreased when 300 mM sucrose was included in the medium. In contrast, nitrogen concentration of embryos was lowest in medium containing 150 mM sucrose and increased with lower sucrose concentrations (1.5 or 15 mM). Both nitrogen content and concentration were greatest for embryos grown in medium containing the highest glutamine concentration (120 mM) and lowest in the lowest glutamine concentration (0.6 mM).

Accumulation of protein and oil varied according to the concentration of carbon and nitrogen available suggesting that embryo composition is at least in part controlled by the mother plant.

**P-1072** Antioxidant Levels And Activities Of Related Enzymes In Carnation Plantlets During Thidiazuron-Induced *In Vitro* Hyperhydricity. D. Sankhla, S. Trivedi, TIM D. DAVIS, and N. Sankhla. Texas A&M University Research and Extension Center, 17360 Coit Road, Dallas, TX 75252.

Nodal explants from *in vitro* grown carnation 'German Red' plantlets were placed on MS medium containing 5  $\mu$ M naphthaleneacetic acid with or without 1  $\mu$ M thidiazuron (TDZ). On the medium containing TDZ,

profuse shoot regeneration occurred, but intense hyperhydricity and callusing were observed within 4-5 weeks. In comparison to the normal control plants grown without TDZ, the glassy and translucent hyperhydric plantlets exhibited lower activities of catalase and superoxide dismutase. Although initially there were no differences in peroxidase activity, as time progressed a sharp increase in the activity of this enzyme was observed in the hyperhydric tissue. As hyperhydricity progressed with time, a concomitant decline was observed in the activity of ascorbate peroxidase. Similarly decreases in the activities of monodehydroascorbate reductase and glutathione reductase were detected during the early stages of hyperhydricity. Initially, the activity of dehydroascorbate reductase remained relatively constant, but as time progressed the activity of this enzyme also declined in the hyperhydric tissue. Concentrations of ascorbic acid, glutathione, and proline were much lower in hyperhydric tissue compared to the control. Likewise, lipid peroxidation was less in the hyperhydric tissue compared to the control. These results indicate a lower level of protection against active oxygen species and hydroperoxide-dependent oxidations in the hyperhydric plantlets.

**P-1073** Microscopical Observation Of Cultured Tissues of Plant. Y. TAHAMA. Laboratory of Plant Pathology, School of Bioresources, Hiroshima Prefectural University, Shobara 727, Japan.

In my previous reports, it was shown that the cultured cell suspension of carrot proceeded to change into non-transparent bodies and belt-like body; and the protoplast of tobacco changed into hypha-like bodies and belt-like body. The results of these experiments were as follows. The specimen of carrot on the cover glass was stored in 5° C refrigerator from November 22, 1990, to October 20, 1992. Experiment was started on January 12, 1993, using an optical microscope instead of cell processor with laser. Accumulation of fundamental round bodies and rubber boat-like bodies composed of black color of the central part and white color of the outer part were observed. And, they proceeded to change into septate mycelium-like bodies which were of a white color and the outer part of which was enveloped by a black color, just like mycoplasma. The specimen of protoplast of tobacco was stored in 5° C from November 27, 1990, to December 3, 1992. Observation was started on January 7, 1993. Aseptate mycelium-like bodies, conidiophore-like bodies and conidiospore-like bodies were observed. They were composed of white color of the inner part and black color of the outer part. The conidiospore-like bodies seemed to be a kind of fundamental style of round bodies of mycoplasma. Nipples, crinkled films and spots were observed, just like mycoplasma.

**P-1074** Use Of Tissue Culture Techniques To Improve Louisiana Soybeans. S.S. CROUGHAN. Rice Research Station, Louisiana Agricultural Experiment Station, L.S.U. Agricultural Center, Crowley, LA 70527.

Tissue culture techniques are being used to develop germplasm for use in soybean varietal improvement. Somaclonal variants are derived from immature cotyledons. All germplasm sources have responded to either a 2,4-dichlorophenoxyacetic acid and/or a naphthaleneacetic acid based medium, giving high rates of embryo development. Plants are grown to maturity and seed collected. The progeny is then evaluated for improved resistance to a number of important pests in greenhouse and laboratory tests. Screening methods have been developed to quickly evaluate somaclones for increased resistance to aerial blight. Somaclones are also evaluated for increased resistance to soybean looper. Somaclones that express increased resistance can then be field tested and also used as new sources of germplasm in conventional breeding programs. Another culture methods are being explored for the potential to rapidly produce double-haploids from soybean  $F_1$  material. Pollen-derived callus can be obtained from all germplasm sources tested. Embryo development has been limited. This technique could expedite the development of new cultivars.

**P-1075** Use Of Somatic Embryogenesis For Hybrid Alfalfa Seed Production. A.R. McElroy and L.J. LANGILLE. Agriculture Canada, Plant Research Centre, C.E.F., Ottawa, Ontario, Canada, KIA OC6.

Substantial alfalfa forage yield increases could be realized by using hybrid cultivars rather than the traditional synthetics, which are advanced generation populations derived from a group of parental clones. To produce alfalfa hybrids, the parental clones must be propagated and established in seed production fields. Vegetative propagation using customary methods is not practical. A transplant plug technique was developed that uses somatic embryogenesis to mass-multiply alfalfa plants *in vitro* in a form suitable for transplanting directly into the field (McElroy and Brown, 1992. *Can. J. Plant Sci.* 72:483-485).

Subsequent work has focused on the incorporation of genes for somatic embryogenesis into disease resistant, agronomically superior alfalfa germplasm. The *in vitro* regeneration trait was obtained from diverse sources: A70-34 from cv. 'Rangelander', A53-24 from cv 'Saranac' and F1-1 from wild *Medicago falcata*. Through recurrent selection, it has been possible to increase somatic embryo quality, the frequency of embryogenic genotypes in progeny populations, and vastly improve agronomic characteristics and disease resistance. Elite genotypes should be ready for release to industry beginning in 1994.

**P-1076** *In Vitro* Screening For *Ascochyta* Blight Resistance In Chickpea (*Cicer arietinum* L.). A.K. Sudha Vani and V.D. Reddy. CPMB, Osmania University, Hyderabad-500 007, A.P., India.

Callus cultures of chickpea derived from blight resistant cultivar (ILC 3279) and blight susceptible cultivars (IOC 4973 and PB-7) were exposed to the culture filtrate of the pathogenic isolate Hissar of *Ascochyta rabiei*, the casual agent of gram blight. Callus survival was determined after exposure to various concentrations of the filtrate (25%, 50%, 75%, and 100%).

All the concentrations of culture filtrate retarded growth in all the cultivars. The highest rate of survival was seen in cultivar ILC 3279 (76.16%) on 25% filtrate followed by IOC 4973 (24%) compared to 100% and 92% survival in the respective controls. The survival rate was 58.33% (ILC 3279) and 16% (ILC 4973) on 50% culture filtrate. Where as in cultivar PB-7 all the concentrations inhibited callus growth. Autoclaved culture filtrate did not show any deviation from the control, suggesting the heat labile nature of culture filtrate.

This technique can be effectively employed for screening resistant genotypes in the segregating/mutagenised populations.

**P-1077** Screening Of Four Rice Cultivars At Cellular Level Against *Xanthomonas oryzae* pv. *oryzae*. RAVINDEDR KAUR, B.S. Thind, S.S. Gosal, and J.N. Gupta. Department of Plant Pathology, Punjab Agricultural University, Ludhiana-141004, India.

The calli induced from four rice (*Oryza sativa* L.) cultivars (i.e. Pusa Basmati, 1, Taichung Native-1, PR-106 and PR-111 on modified MS medium) were used for screening against *Xanthomonas oryzae* pv. *oryzae*. Three isolates (i.e. highly virulent, moderately virulent, and avirulent of *X. oryzae* pv. *oryzae* isolated from PR-106) were used. The culture filtrate of the bacterium obtained after 72 hours of incubation at  $27 \pm 1^\circ \text{C}$  in nutrient broth kept on shaker (120 rpm) was filter sterilized and mixed with  $\text{MSP}_{0.2}$  medium (MS supplemented with 2 mg/l of 2,4-D and 0.2 mg/l of BAP) in the ratios of 4:1, 3:1, 2:1, 1:1, 0:1, 1:2, 1:4, 1:8, and 1:12. Four- to five-month-old embryogenic calli (E-calli) were exposed to aforesaid ratios of culture filtrate and  $\text{MSP}_{0.2}$  medium and kept at  $26 \pm 1^\circ \text{C}$  in the dark for 25 days. All the ratios above 1:2 inhibited the growth of calli derived from all the cultivars, except cv. PR-111. The calli tolerant to culture filtrate were transferred onto  $\text{MSP}_{0.2}$  medium for the recovery of growth. After 15 days, the calli showing growth were transferred onto MSB medium (MS salts with 3 mg/l kinetin and 0.5 mg/l of NAA) for regeneration of plantlets. The ratio 1:2 inhibited the regeneration potential in all the cultivars tested. However, ratio 1:4 exhibited inhibition of regeneration potential of only Pusa Basmati-1, Taichung Native-1 and PR-106.

- P-1078** Production Of Thermostable Amylolytic Enzymes From *Clostridium* sp. M.V. SWAMY and G. Seenayya. Department of Microbiology, Osmania University, Hyderabad-500 001, India.

At present maltose producing amylases are obtained from higher plants and they are not thermostable. Production of thermostable maltose and maltooligosaccharide producing amylases from microbial sources has a high potential for the commercial exploitation. Therefore, production of thermostable enzymes from thermophilic bacteria have a great significance. Cell extracts of *Clostridium* sp. an anaerobic thermophilic bacterium, contained these type of amylase activities. These enzymes had temperature and pH optima of 65° C and 6.0, respectively. These enzymes were largely cellbound during the growth of the organism with 0.5% starch. On increase in starch content of the growth medium, the excretion of amylolytic enzymes into culture broth have increased and however a decrease in the total activity was observed. The growth of the microbial cells for the increased amylase production was more on maltose and strongly repressed by glucose. The details of the enzymes regulation and localization aspects are discussed.

- P-1079** Effect Of Hormones On Callus Of *Mikania* sp. A. CERDEIRA, E. Gardini, A. Pereira, and S. Franca. EMBRAPA C.P.69, Jaguariúna, Sp, 13820, Brazil.

Plants are known to produce complex secondary metabolites needed for their protection. Those compounds are also an important source for the making of pharmaceutical and insecticides. Callus and root cultures may be a viable source of material for producing secondary compounds. *Mikania* sp. is a medicinal plant utilized in Brazil. Extracts from leaves are utilized as anti-inflammatory. Coumarin is the metabolite responsible for these effects, also acting at the nervous system with sedative and hypnotic action. The objectives of this research were to determine if callus, micropropagated plants and root cultures were a suitable source to produce coumarin.

Several concentrations of NAA (naphthaleneacetic acid) or 2,4-D (2,4-dichlorophenoxyacetic acid) and both in combination were tested for callus induction, maintenance and root cultures. Callus and root cultures were affected by the source of explants, media type and growth regulators concentrations. Root cultures differentiated from callus tissue in B5 media with supplements of 0.5 mg NAA l<sup>-1</sup> or 0.5 mg 2,4-D l<sup>-1</sup>. Higher concentrations of 2,4-D induced more callus growth. Presence of coumarin was detected by TCL in callus and root cultures.

- P-1080** Effect Of Auxins And Cytokinins On Anthocyanin Production In Cranberry Cell Cultures. D.L. MADHAVI and M.A.L. Smith. University of Illinois, Urbana, IL 61801.

Growth regulators play a significant role in triggering cell differentiation and secondary metabolite formation

in plant cell cultures. Production of anthocyanins in cranberry cell cultures was modified by altering the growth regulators in the culture medium. Zeatin significantly promoted anthocyanin production either singly or in combination with IAA or NAA. 2,4-D in general had an inhibitory effect on anthocyanin production. The effect of growth regulators on the anthocyanin profile and the expression of phenylalanine ammonia lyase activity, one of the key regulatory enzymes in the anthocyanin biosynthetic pathway were investigated.

- P-1081** Thin Cell Layer Culture Of A Monocot, *Setcreasea pallida* (Syn. *Tradescantia pallida*) cv. Purple Heart. DOUGLAS W. DARNOWSKI<sup>1</sup> and E.D. Earle<sup>2</sup>. <sup>1</sup>Section of Plant Biology and <sup>2</sup>Department of Plant Breeding, Cornell University, Ithaca, NY 14853.

Thin cell layer culture (TCL), which uses explants composed of epidermal and subepidermal cells placed on defined media, provides a useful regeneration and bioassay system for the production of roots, shoots, callus, and, in some cases, flowers. Such floral regeneration has been observed in species from several different plant families. Almost all TCL work to date has involved dicots, principally tobacco. Because of the potential value of a monocot TCL regeneration system, we have begun work on TCL culture of the monocot *Setcreasea pallida* (syn. *Tradescantia pallida*) cv. Purple Heart (*Commelinaceae*), which contains anthocyanin pigments with potential economic value. To date, peeled 0.2 x 1.0 cm explants from subfloral internodes containing 1-4 layers of epidermal and subepidermal cells have produced a friable, off-white callus on several modified LS media supplemented with auxin alone or with cytokinin. Work in progress includes establishment of suspension cultures from this callus and further experiments on organ regeneration.

- P-1082** Theanine Formation By Tea Cells. T. Takihara, I. Sakane, and T. KAKUDA. ITOEN Ltd., Central Research Institute, Shizuoka 421-05, Japan.

Theanine (THE:  $\gamma$ -glutamylethylamide), an amide that enhances the taste of infused green tea, was first found in shoot tips of tea plant (*Camellia sinensis* L.). The compound was known to have biological effects, which are an inhibitory effect against increasing spontaneous motor activity of mice dosed with caffeine and lowering effect against the high blood pressure. However, THE content in tea leaves is about 1.5% of dry weight and varies in seasons. THE is biosynthesized from glutamic acid and ethylamine (EtNH<sub>2</sub>) catalyzed by THE synthetase. Then we have been studying the culture conditions for biosynthesis of THE in cell culture of tea. The callus was induced from stem of *C. sinensis* cv. Yabukita. THE accumulation in the cultured cells was remarkably increased when ethylamine hydrochloride was added to the modified MS medium and its optimum concentration was 25 mM. The optimum incubation temperature was 25° C, and dark culture was better than the illuminated culture. The amount of THE accumulated in the cells was remarkably increased in culture with the medium supple-

mented with 40 mM of  $\text{NO}_3^-$  and without  $\text{NH}_4^+$ . Finally, THE content in the cells was reached more than 20% of dry cell weight.

- P-1083** Metabolism Of Carbofuran In Cell Suspension Cultures Of Sugarcane As A Function Of Concentration And Incubation Time. SATWANT KAUR, B. Singh, S.S. Gosal, and R.L. Kalra. Department of Entomology, Punjab Agricultural University, Ludhiana-141004, India.

The metabolism of  $^{14}\text{C}$ -carbofuran in sugarcane (*Saccharum officinarum* L.) cell suspension cultures was investigated as a function of incubation time and concentration of insecticide. Cell suspension cultures were incubated with  $^{14}\text{C}$ -carbofuran (2,2-dimethyl-2,3-dihydrobenzofuran-7-N-methylcarbamate) at 25° C for 48 hours and samples were collected at different intervals of time. Samples were extracted, cleaned up, and radioactivity was assayed using liquid scintillation counter. The identity of metabolites was confirmed using thin-layer chromatography (TLC-autoradiography). The metabolism of carbofuran was found to be changed with the increase of concentration and incubation time of insecticide. An increase in incubation time was found to decrease the total amount of carbofuran and its organic soluble metabolites with an increase in the amounts of water-soluble metabolites. The details of investigations carried out shall be discussed.

- P-1084** Morphogenetical Regulation Under The Influence Of Phytohormones In *Cuminum cyminum*. ANJU DAVE and Amla Batra. Lab. No. 5, Botany Department, University of Rajasthan, Jaipur 302004, India.

*Cuminum cyminum* is a popular spice used for flavoring; however, its essential oil component has even more significant properties with its stimulating, stomachic, astringent, and cooling effects. Phytohormones like auxins and cytokinins were used while culturing cumin tissues. Hypocotyl explant was the best source material for callus production. Specific auxins like IAA and IBA were effective to induce rhizogenesis, while 2,4-D induced mere callusing, in the hypocotyls inoculated on solid Murashige & Skoog's (MS) medium. However, NAA was found to be effective in greening of callus with rhizogenesis. Cytokinins like BAP and Kn were found to be more favorable for callus production. Auxins and cytokinins (BAP-NAA), when tried in combination, made the callus nodulated. Effect of phytohormones in combination (BAP-IAA-IBA, 5 mg/l each) induced significant effect, leading to differentiation in the callus. Field trials are going on to make the plants grow and survive.

- P-1085** Rapid Transformation Of Eggplant (*Solanum melongena* L.) Using *A. tumefaciens* Carrying A Binary Vector Containing The Coat Protein Gene From Cucumber Mosaic Virus (CMV). J.F. REYNOLDS, A. Wilkerson, D. Tricoli, P. Russell, H. Quemada, R. McMaster, S. Mastenbrook, and L. Herrygers. EPG, Asgrow Seed Company, Kalamazoo, MI.

Eggplant is a vegetable cultivated in Europe as well as the far east. Production of the crop is compromised by many fungal and viral diseases. A method of overcoming many of these diseases would be through the introduction of engineered genes known to cause resistance. This report describes the use of somatic embryos to rapidly produce transgenic eggplant containing the engineered CMV viral coat protein gene. We cocultured cotyledon explants from *in vitro* germinated seeds with *A. tumefaciens* containing a binary vector with the CMV coat protein and NPT II genes. Following coculture, cotyledon explants were transferred to medium with 100 mg/l Kanamycin. After 2 weeks on 100 mg/l Kanamycin, callus was observed on the explants. Somatic embryos developed from these calli after an additional week culture. Embryos could be germinated by culturing on MS medium without growth regulators. Germinated embryos were isolated to identical medium and allowed to develop for an additional 1 to 2 weeks. Analysis of  $\text{R}_0$  plants showed a positive NPT II signal as well as a positive PCR signal by probing with sequences from the NPT II and CMV coat protein genes. We have demonstrated that transformed eggplant can be produced via somatic embryogenesis. Total time from coculture to potted plant was 2 months.

- P-1086** *In Vitro* Tumor Formation On *Psidium guajava* Infected By *Agrobacterium tumefaciens*. O. VAZQUEZ-MARTINEZ, J.L. Moreno-Hernandez, and L.L. Valera-Montero. Dpto. Fitotecnia, C. Agropecuario, UAA. Ave. Universidad 2100, C.P. 20100, Aguascalientes, Mexico.

Genetic engineering may be helpful on guava breeding programs since some advances on *in vitro* culture of guava have been achieved and possibly this plant may be susceptible to genetic transformation under these conditions. Unfortunately, there is a lack of information on this topic, and therefore this work was oriented to evaluate the susceptibility of guava *in vivo* and *in vitro* to be infected by *A. tumefaciens* strain C58 through the observation of tumors and nopaline assays. Guava and *Kalanchoe tubiflora* plantlets 15-20 cm tall were grown on pots under 16 hours of photoperiod, 20-25° C and 90% RH. Plantlets were inoculated with an overnight culture of *A. tumefaciens* C58. In order to produce wounds, 240 guava plantlets were punctured by using a disposable insulin-syringe, and 240 were decapitated. The same procedure was applied to 150/150 *Kalanchoe* plantlets. All plants were kept under the original conditions for six months. For the *in vitro* experiments, 100 axenic leaf discs from young guava plants and 60 tobacco (Xanthi) leaf discs were cocultivated with an overnight culture of C58 during 24 hours. Furthermore,

axenic hypocotyls were cocultivated during 3 hours with  $1 \times 10^7$ ,  $1 \times 10^8$ , and  $1 \times 10^9$  bacteria/ml using 175 explants for each concentration. Subsequently, explants were placed on MS supplemented with ascorbic acid 100 mg/l, citric acid 150 mg/l, and carbenicillin 500 mg/l. Explants were kept at 22-25° C and 16/8 photoperiod. Tumor induction *in vivo* was negative in guava since no tumor or visible signs of overgrowth were apparent, unlike *Kalanchoe* having 98% punctured and 57% decapitated plantlets with tumor formation. On the other hand, 40-49% guava and 63% tobacco leaf discs formed tumor *in vitro*. Guava hypocotyls showed 66.8%, 68.5%, and 34.8% of explants with tumor formation for bacterial concentrations of  $1 \times 10^7$ ,  $1 \times 10^8$ , and  $1 \times 10^9$ , respectively. Finally, all of the tumors tested showed the presence of nopaline.

**P-1087** Genetic Transformation And Transgenic Plant Regeneration Of Grapevine. L. MARTINELLI and G. Mandolino. Laboratory of Biotechnology, Istituto Agrario, I-38010 San Michele all'Adige (TN), Italy.

Species of the genus *Vitis* are particularly difficult to be transformed since stable transgenic grapevine has not been reported so far. Regenerative capability has been described as the most crucial problem affecting transgenic grapevine. However, we established a suitable protocol for genetic transformation and transgenic plant regeneration on somatic embryos from petiole-derived callus cultures of *Vitis rupestris* Scheele. The coculture of individual embryos during the induction of secondary embryogenesis with *Agrobacterium tumefaciens* strain LBA4404 which contains the plasmid pBI121 carrying the neomycin-phosphotransferase and the  $\beta$ -glucuronidase genes, produced transformed cellular lines competent of recurrent somatic embryogenesis. Precocious selection on high levels of kanamycin was an important part of our transformation protocol. Our system proves to give a good genome stability of the inserted genes and a low level of somaclonal variation: the corollary is the proper overlap between transgenic and regenerating cells. Isolated embryos were capable to regenerate plants with a relevant efficiency (13%). As a result, 47 different plant clones were obtained from distinct transformation events. Both somatic embryos and regenerated plants proved to be stably transgenic since histochemical and molecular assays exhibited positive responses. Moreover, no chimeric tissues were detected. Molecular tests demonstrated no loss of the GUS gene following either the long-term embryo cultures and the plant regeneration events.

**P-1088** Bio-Technological Aspects Of *In Vitro* Multiplication Of Certain Bio-Mass Yielding Plants. CH. AYODHYA RAMULU and D. Rao. Department of Botany, Kakatiya University, Warangal-506 009, India.

Biotechnology offers a number of non-conventional approaches that may supplement the conventional methods of crop improvement. Realizing that the resources of the earth are not infinite, the prospects of exploiting new and renewable sources of energy through the use of bio-

technology should receive increased attention. The main intent of clonal propagation is to establish plants that are uniform and predictable of selected qualities. In view of the superior rate of planting material required, interest in the use of tissue culture as an alternative to traditional asexual multiplication. Establishment of callus cultures from various explants of forest tree species *Acacia auriculiformis* and *Albizia lebbels* were obtained on the B5 medium supplemented with 2.75 mg/L, 2,4-D and 1.50 mg/L kinetin. The induced calli were maintained with regard to various physical factors like light, temperature, and humidity have been standardized.

Among the various explants used for direct morphogenesis of explants like leaf, petiole, short apex, seeding roots terminal, axillary buds and hypocotyl segments inoculated for direct shoot bud induction. Plantlet formation via shoot bud formation is achieved efficiently with hypocotyl explants by combination of 2.50 mg/L and 1.75 mg/L kinetin and maximum frequency (62-74%) of buds obtained and separated them and inoculated on rooting medium. These plantlets are ready for propagation as planting material is in progress.

**P-1089** Somatic Embryogenesis And Plant Regeneration In Cassava (*Manihot esculenta* Crantz.). T.C. NARAYANASWAMY, N.M. Ramaswamy, and S.R. Sree Rangaswamy. Centre for Plant Molecular Biology, TamilNadu Agricultural University, Coimbatore-641 003, India.

Investigations on *in vitro* culture studies in Cassava (*Manihot esculenta* Crantz.) were carried out with the objective of inducing callus and regenerating plants for genetic variability. Two Cassava cultivars viz. CO 2 and CO 3 were evaluated for their ability to induce callus and regeneration. The explants used were shoot tip, axillary bud, stem segment, and young leaf lobes. Murashige and Skoog (MS) medium supplement with 2,4-D (6 mg/l) gave maximum callus induction from shoot tip and young leaf lobe explants followed by axillary bud and stem segments. 2,4-D at 4 to 6 mg/l promoted friable and embryogenic calli in all the explants tried. The shoot tip callus of CO 2 on transfer to MS medium with 2,4-D (0.01 mg/l) + BAP (0.1 mg/l) + GA<sub>3</sub> (1.0 mg/l) differentiated into clusters of somatic embryos after four weeks. Long-term cultures were established and maintained up to one year by repeated subculturing of proliferating embryogenic callus. Regenerated plantlets further developed after eight weeks of somatic embryos transferred to hormone free medium and were cultured on half MS for subsequent development of plants and for root formation. The regenerants have been transferred to the soil for field establishment.

**P-1090** Anther Culture Response Of Wheat and Wheat x Wheatgrass Hybrids. H.C. Sharma, O. Benhabib, H.W. Ohm, and C.S. Lu. Purdue University, Department of Agronomy, West Lafayette, IN 47907.

Anther culture response of 22 wheat cultivars studied was genotype dependent for callus induction (0-12%) and plantlet regeneration (0-35%). Microcallus

formation was observed in some genotypes in some of the anthers that were plated with only one locus in contact with the medium indicating a relationship of anther orientation to its response to microcallus formation. Application of anther culture technique was extended to wheat x wheatgrass hybrids. Up to 2% callus induction response was realized from anther culture of intergeneric F1 hybrids between wheat (*Triticum aestivum*) and wheatgrass species (*Agropyron elongatum*, *A. intermedium*, and *A. trichophorum*). Complete pollen sterility of these hybrids could largely be responsible for their low response to anther culture. Work with backcrosses of wheat x wheatgrass wide crosses is in progress.

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**P-1091** Efficient Plant Regeneration From Callus Cultures Of Mulberry Trees. Y. SAHOO, S.K. Patnaik, and P.K. Chand. Tissue & Cell Culture Facility, Post-Graduate Department of Botany, Utkal University, Bhubaneswar-751004, Orissa, India.

Young internodal segments were excised from 3-year-old mulberry trees (*Morus indica* var. S-1635, S-34) and were inoculated onto MS medium with different concentrations and combinations of phytohormones and other growth adjuvants like coconut milk (CM) and casein acid hydrolysate (CH). Callusing was best observed on MS medium fortified with 2,4-D (2.0 mg/l) and BAP (0.5 mg/l). Addition of CM and CH either individually or in combination to the above medium did not show any significant stimulatory effect on callus induction. Nevertheless, the proliferation of mulberry calli needed the presence of BAP alone (0.5 mg/l) along with CM (15%) and CH (100 mg/l). Development of green shoot buds from the calli was achieved within 20-25 days when transferred either to MS + BAP (0.3 mg/l) or MS + CM (15%) + CH (100 mg/l). The frequency of shoot bud formation was faster and higher on the former medium. An average of  $4 \pm 1$  shoot buds was recorded per callus. Three 4-week-old calli were found to be ideal for shoot bud induction. The older the calli, poorer was the morphogenetic potential. Rhizogenesis was elicited within 8-10 days in MS medium with IBA (1.0 mg/l). Complete plantlets with a well developed shoot and root system were recovered within 8 weeks following callus induction. Some of these plants were successfully transferred to field conditions with <10% mortality.

**P-1092** Regeneration Studies In Pigeonpea Varieties Of Different Maturity Groups. PARAMPREET KAUR and J.K. Bhalla. Cytogenetics and Tissue Culture Laboratory, Department of Botany, Osmania University, Hyderabad 500 007, India.

A comparative study was made to test the regeneration ability of shoot tip, stem, leaf, and cotyledon explants from young seedlings of two cultivars of Pigeonpea: Viz Hy3C, a late maturity type, and ICPL 89021, an early maturity type. The explants were selected from asepti-

cally grown seedlings and cultured on MS and Blaydes media. Different hormone adjuvants such as 2,4-D, 2,4-D + BAP, NAA, NAA + BAP and BAP were tested. The study was undertaken to find out whether there was a differential response between cultivars belonging to different maturity groups. Of the auxins tested, 2,4-D was found to be the most effective for callus induction, but the supplementation of BAP at lower levels had a promoting influence on callus growth. Regeneration was obtained from the stem explants (3-5 mm) obtained from young seedlings cultured on MS medium supplemented with NAA + BAP. Complete plants have also been obtained either by direct regeneration of the excised segments of cotyledons and shoot tip or through the differentiation of callus cultures of the same explants. However, cotyledons were the most suitable source for obtaining callusing and regeneration in both the varieties of Pigeonpea. The results obtained from this study provide a scope for micropropagation of superior genotypes obtained through hybridization between early and late maturity groups of Pigeonpea since the regeneration ability of both maturity groups was similar.

**P-1093** Cassava Root Thickening *In Vitro*. D.W. DARNOWSKI<sup>1</sup> and E.D. Earle<sup>2</sup>. <sup>1</sup>Section of Plant Biology and <sup>2</sup>Department of Plant Breeding, Cornell University, Ithaca, NY 14853.

Cassava (*Manihot esculenta*, Euphorbiaceae) is a major dietary staple for much of the world's population. The edible crop is the secondarily thickened roots, which are rich in starch. Due to the importance of cassava roots for human nutrition, information concerning their secondary thickening is of great agronomic value. Using cultivars TMS 30337 and TMS 30572, provided by Dr. S.K. Hahn (International Institute of Tropical Agriculture, Ibadan, Nigeria), we have established a culture system which yields miniature thickened roots with gross morphological similarity to *in vivo* thickened roots. This similarity is maintained, and the size of the thickened roots is increased, in at least some *in vitro* plantlets transferred to soil. Starch deposition has not yet been observed near control levels. We have also established a vertically-oriented plant-free root culture system, based on the radish root culture system of Loomis and Torrey (1964; *PNASUSA*. 52:3-10) which displays a similar thickening in response to BA (1-5  $\mu$ m) whether or not NAA (5  $\mu$ m) is present. This response occurs for roots of both radish and cassava. In the case of cassava the thickening is morphologically similar to that observed in the roots of *in vitro* plantlets. This system allows easy manipulation of these roots. The authors will also present a poster on *Setcreasea*.

**P-1094** Different Embryogenic Competence Within Single Explants Of *Camellia Japonica*. M.C. PEDROSO and M.S. Pais. Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Bloco C2, Piso 1, Campo Grande, P-1700 Lisboa, Portugal.

Several *in vitro* culture systems have been developed to study somatic embryogenesis in *Camellia ja-*

*ponica*. Experiments on direct embryo formation in single leaves indicate that cells from well defined leaf-regions possess different morphogenic competencies. Embryo formation (direct and indirect) was restricted to parenchyma cells from leaf periphery. Electron probe X-ray microanalysis (EPMA) showed that embryogenic regions have characteristic spectra already before induction. Histological examination of samples during the induction of direct embryogenesis from leaves, stem and root segments, indicate that not all cells from a potentially embryogenic tissue become competent and that only a few become determined. EPMA and histological studies indicate that cell wall composition from competent cells is different from noncompetent ones. The change occurs shortly after the induction treatment. Similar differences at cell wall level are present in cells from cotyledons (embryogenic tissue). Severe fluctuations in the number of calcium oxalate crystals also occur, but only in induced tissues. Temporal and spatial relationships between these two phenomena and embryogenic competence and determination is not yet fully understood.

**P-1095** Ureide Metabolic Pathway In  $N_2$  Fixing Pigeonpea (*Cajanus cajan* L.). D. SREENIVAS REDDY, Gopal Reddy, and H. Polasa. Department of Microbiology, Osmania University, Hyderabad-500 007, India.

The ureides, allantoin, and allantoate are important nitrogenous metabolites of symbiotic  $N_2$  fixation in tropical legumes. Ureides are synthesized in root nodules and transported to various parts of the plant tissues. Ureides are metabolized either by allantoicase or allantoate amidohydrolase pathway in plant tissues and serve as primary source of N for protein synthesis. Acetohydroxamate (AHM), a specific inhibitor of urease, was used as probe to determine ureide metabolic pathway. AHM induced severe nitrogen deficiency in  $N_2$  fixing pigeonpea (ureide plant) while alfalfa (amide plant) was unaffected. AHM induced chlorosis, reduced dry weight and total nitrogen contents were observed. The inhibitory effects were overcome by application of combined nitrogen. Allantoinase and urease activities were markedly reduced. Consequently, ureides and urea were accumulated in all parts of the plant tissues of pigeonpea. These inhibitory studies indicated that ureides are catabolized via allantoicase-urease pathway in pigeonpea plant tissues.

**P-1096** Rapid Micropropagation Of *Dalbergia sissoo* Roxb. V.A. Chauhan, P.C. Josekutty, G. Prathapasenan. Department of Botany, Faculty of Science, The M.S. University of Baroda, Baroda 390002, India.

Rapid micropropagation of *Dalbergia sissoo* Roxb. was achieved through *in vitro* techniques by the proliferation of axillary buds from 30- to 40-year-old plants. Bud break was achieved within six days when cultured on MS medium supplemented with kinetin (2 to 5 ppm), Indole butyric acid (0.1 to 0.5 ppm) and Benzyl adenine (2 to 5 ppm). Elongation and multiple shoot formation took place on MS medium with reduced concentration of

salts and kinetin within 15 days. Rooting of these shoots were readily obtained in MS medium supplemented with Naphthalene-acetic acid and Indole butyric acid in less than 5 days.

**P-1097** Cloning Of 20-Year-Old Female Jojoba (*Simmondsia chinensis* [Link.] Schneider). R.R. SINGH\*, T.S. Rathore, S. Rama Rao, and N.S. Shekhawat. Plant Biotechnology Laboratory, Department of Botany, J.N.V. University, Jodhpur-342 001, India.

Jojoba was introduced in India during early Seventies. It is becoming a popular oil crop in arid and semi-arid regions of the country. However, saplings of suitable tested female plants are not available for plantation. The present investigation was initiated to develop tissue culture technology for cloning of female plants. Nature of explant, season of explant collection, the culture medium, cytokinin used and incubation conditions influenced the establishment of cultures and their *in vitro* multiplication. Explants were harvested from 20-year-old female plants of jojoba growing in the experimental field of National Bureau of Plant Genetic Resources (NBPGR), Jodhpur, India. From a single nodal shoot segment, 8-10 shoot buds differentiated after 12-15 days of culture at  $28 \pm 2^\circ$  C on Murashige and Skoog's (MS) medium containing  $0.1 \text{ mg l}^{-1}$  IAA +  $2.0 \text{ mg l}^{-1}$  BAP + additives (Adenine sulphate,  $25 \text{ mg l}^{-1}$ ; Arginine,  $25 \text{ mg l}^{-1}$ ; Ascorbic- and Citric acids,  $25 \text{ mg l}^{-1}$  each) under mixed light of  $30 \mu\text{Em}^{-2}\text{s}^{-1}$  photon flux density (12 h/day photoperiod). The shoots were multiplied *in vitro* by (1) subculturing of *in vitro* produced shoots and (2) repeated transfer of mother explant on MS medium supplemented with  $0.1 \text{ mg l}^{-1}$  IAA +  $1.0 \text{ mg l}^{-1}$  BAP + additives. About 70% of the IBA ( $25 \text{ mg l}^{-1}$  in 1/4 strength MS liquid medium for 1 h) pulse treated shoots rooted within 10-15 days on hormone-free 1/4 strength MS medium supplemented with activated charcoal. ( $500 \text{ mg l}^{-1}$ ). Initial dark incubation for 4 days at  $33 \pm 2^\circ$  C was found to be essential for early root induction. The cloned plants were cytologically evaluated. It is suggested that protocol developed can be used for cloning of selected jojoba plants in Indian desert.

\*Senior author is recipient of Research Associateship from CSIR, New Delhi.

**P-1098** Rapid Micropropagation Of *Ficus religiosa* L. S.R. Deshpande, P.C. Josekutty, and G. Prathapasenan. Department of Botany, Faculty of Science, The M.S. University of Baroda, Baroda 390002, India.

Rapid multiplication of *Ficus religiosa* was achieved *in vitro* from juvenile nodal explants, collected from a mature tree of the species. The apical and the axillary buds, dormant on these nodal explants, sprouted, when cultured on MS medium supplemented with 2 to 5 ppm BA and 0.5 to 2 ppm IBA. Multiple shoot formation was induced when these sprouted shoot buds were transferred to MS medium supplemented with a reduced concentration of BA and 1 to 2 ppm adenine sulphate. Roots were induced at the cut ends of these regenerated shoots on MS medium supplemented with synthetic

auxins, 0.1 to 1 ppm NAA and 0.5 to 2 ppm IBA. Proliferation of these roots was achieved on transfer to MS medium with half the strength of macro- and micro-nutrients.

**P-1099** Micropropagation Of Grapes (*Vitis vinifera* L. cv. Thompson Seedless). A.N.S. GOWDA and A. MURALIKRISHNA. Biotechnology, TNAU, Coimbatore 641 003, India.

Axillary bud explants excised from 11th, 12th, and 13th nodes of canes (grapevine) gave the highest establishment percentage of 36%. But those collected from very tender vines (2-4 nodes) as well as very old portion (23-25 nodes) failed to establish in *in vitro*. Profuse multiple shoot initiation occurred from axillary bud explants on MSB containing BAP alone and BAP-KN combinations rather than kinetin alone. But the shoots produced on MSB supplemented with BAP resulted in high compressed, small shoots. The maximum number of 12.33 multiple shoots per culture was obtained on MSB supplemented with BAP 1.0  $\mu$ M plus KN 1.0  $\mu$ M. This was closely followed by the treatment containing BAP at 2.0  $\mu$ M concentration and produced 10.67 multiple shoots per culture. Internodal stem segments of grape responded positively to callus, the rapid callus initiation was obtained in only 10 days on MSB with NAA at 1.0  $\mu$ M concentration. The highest number of roots per shoot was 8.15 on MSB with 1.0  $\mu$ M of IBA, whereas NAA at 1.0  $\mu$ M concentration was able to induce an average of only 3.56 roots per shoot. Grape shootlets showed favorable response for hardening under an illumination of 5,000 1x recording 34% survival.

**P-1100** Micropropagation Strategy For Guava (*Psidium guajava* L. cv. Sardar). A.N.S. GOWDA and A. MURALIKRISHNA. Biotechnology, TNAU, Coimbatore 641 003, India.

Polyphenol oxidation was relatively minimized by frequent transfers on 1/2 strength Murashige and Skoog's basal medium (30% survival). Shoot tips needed fewer frequent transfer than leaves. About 10% of cultures were able to initiate callus on MSB containing 0.5 mg/l<sup>-1</sup> NAA. Development of organs in guava leaf callus was very negligible. However, the maximum number of multiple shoots per culture was only 3.10 on MSB, containing BAP at 4.0 mg/l<sup>-1</sup>. The shootlets produced thus also grew very slowly and were not able to reach Stage III for *in vitro* rooting. Further kinetin was more favorable than BAP for adventitious shoot initiation in shoot tip explants. An average of 2.00 multiple shoots per culture were produced on MSB containing KN 1.0  $\mu$ M concentration. The maximum number of roots per shootlet was 2.85 on MSB supplemented with IBA at 1.0  $\mu$ M concentration. Hardening of guava plantlets with an illumination of 10,000 1x resulted in better survival of 56% than those with 5,000 1x which showed 42% survival. Equal volumes of sand plus soil as potting mixture achieved the highest survival rate of 50%.

**P-1101** Breeding Tomato Plants For Regenerability *In Vitro*. N.M. Piven, M. Sánchez, D. Infante. Instituto Venezolano de Investigaciones Científicas (IVIC) Núcleo Experimental de Biotecnología Agrícola (N.E.B.A.), Apartado 21827, Caracas 1020-A, Venezuela.

A number of researchers report successful selection of plant genotypes for increased ability to regenerate plants *in vitro*. The authors suggest that different genotypes in tissue culture have a difference in morphogenetic capacity. We tested 9 different commercial varieties of cultivated tomato *Lycopersicon esculentum* L. for the plant regeneration *in vitro*. It includes a few aspects of regeneration of plants *in vitro* from different genotypes, explants as well as calli, bud and shoot formation, rooting and transition to *ex vitro* conditions. In general, two different medium were used in these experiments: 1) Zeatine, Indolyl acetic acid (IAA), Gibberelline (GA<sub>3</sub>) and 2) Benzylaminopurine (BAP), IAA, GA<sub>3</sub>. We found that regeneration efficiency from hypocotil, cotyledones and leaves of different genotypes was best on medium with zeatine. At first, 8 of 9 genotypes regenerated in this medium better. The high regeneration ability was found for the hybrid lines NEMA 512, KADA and varieties Santa Clara and Rio Grande. This plant material can be used for the transformation experiments more successfully than others.

**P-1102** Influence Of Culture Medium Strength And Growth Regulators On The Micropropagation Of *Dioscorea* Yams. S.A. MITCHELL and H.N. Asemota. Biotechnology Centre, University of the West Indies, Jamaica.

Yams (*Dioscorea*) are a leading source of calories for millions of people in the tropical and subtropical regions of the world. Intensive studies aimed at developing a commercial micropropagation system for *Dioscorea* yams have been undertaken in our laboratory. Significant understanding into the physiology of the yam plant has been obtained. Optimal survival of meristem tips of *D. trifida* was obtained on full-strength Murashige and Skoog culture medium supplemented with either 0.1 mg/l 6-benzyl amino purine (BAP) and 0.01 mg/l indole butyric acid or 0.2 mg/l BAP and 1.0 mg/l naphthalene acetic acid producing plantlets by 28 weeks. Initiation of nodal segments was found to be growth regulator specific, with better shoot growth being obtained with BAP than kinetin. The sensitivity of the explants to growth regulators was affected by photoperiod, type and source of nodal explants. For *D. trifida*, establishment under *in vitro* conditions was also affected by type and source of explant which influenced the combination of growth regulators needed to produce optimum results. Growth rate for *D. cayenensis* was improved by addition of ammonium nitrate to the medium. High multiplication rates were obtained for three yam cultivars: *D. Alata* cv. Sweet Yam, *D. cayenensis* cv. Black Wist Yellow Yam, and *D. rotundata* cv. Negro Yam. Tuber yield of *D. cayenensis* and *D. trifida* was the same under both *in vitro* and *in vivo* conditions of hardening.

- T-1001** Development Of An In-Vitro Canine Blood Brain Barrier Model. T.R. PIPPERT, D.R. Umbenhauer, and W.W. Nichols. Merck Research Laboratories, Department of Genetic and Cellular Toxicology, West Point, PA 19486.

The blood brain barrier (BBB) is characterized by the selective passage of compounds across the vascular wall of brain capillaries. We have developed an in-vitro model for the BBB utilizing cultured canine brain microvessel endothelial cells (cbmecs). Cbmecs were harvested and grown to confluence on collagen/fibronectin coated polycarbonate membranes with 3  $\mu$ m or 12  $\mu$ m pores. The barrier function of the monolayers was assessed by measuring the passage of  $^{14}$ C-sucrose across the cbmecs in a side-bi-side diffusion cell (Crown Glass Inc.). The cbmecs form a barrier to sucrose in-vitro: the rate of sucrose passage (%/min.) across 12  $\mu$ m pore membranes was 0.20%/min. and 0.52%/min. for confluent cbmec and protein coated membranes, respectively, compared to 0.26%/min. and 0.37%/min. for the 3  $\mu$ m pore membranes (cbmec and coated, respectively). The barrier tightens upon addition of increasing amounts of BSA to the assay buffer, resulting in a decrease in sucrose passage across cells grown on both 3  $\mu$ m and 12  $\mu$ m membranes. Using sucrose as an internal leakiness control, the permeability of 18 compounds has been determined using the model. Results indicate that permeability is not necessarily a function of lipophilicity as measured by octanol/buffer partition coefficients. In the case of spiperone, methyl spiperone, and L-689,560, addition of protein (BSA) to the assay buffer decreases the sucrose corrected permeability of the compound, indicating that protein binding may alter the permeability of a compound. Disruption of the cbmec barrier by SDS, arabinose (>1.6 M), and cytochalasin B has also been demonstrated in this model by following the increase in sucrose leakage of treated cells. The model described above could be useful to predict permeability across the BBB and as a screen for compounds that disrupt the blood brain barrier.

- T-1002** Development Of Mammalian Cell Lines Stably Expressing Mouse Prostaglandin Synthase 1 And 2. P.C. CHULADA<sup>1,3</sup>, V.D. Winn<sup>2</sup>, D.A. Young<sup>2</sup>, H.F. Tian<sup>3</sup>, K.R. Tindall<sup>3</sup>, C.D. Loftin<sup>3</sup>, T.E. Eling<sup>3</sup>, and R. Langenbach<sup>1,3</sup>. <sup>1</sup>Toxicology Department, N.C. State University, Raleigh, NC 27695; <sup>2</sup>University of Rochester, Rochester, NY 14642; <sup>3</sup>NIEHS, Research Triangle Park, NC 27709.

Previously, the constitutively-expressed form of prostaglandin synthase (COX1) was believed to be responsible for most biological processes attributable to the prostanoids. However, a second, inducible form of prostaglandin synthase (COX2) was recently identified and may selectively contribute to these processes. To better understand the roles the two isoenzymes play in carcinogenesis/mutagenesis, we have developed derivatives of C3H mouse embryo (10T1/2) and Chinese Hamster Ovary (AS52) cell lines which stably express high levels of mouse COX1 or COX2. A packaged retroviral vector containing each cDNA was infected or

lipofected into 10T1/2 or AS52 cells, respectively. Multiple clones of each cell type containing COX1 or COX2 were selected with G418. Enzyme activities were analyzed by measuring the rate of PGE2 production. A clonal range of activities was seen for each isoenzyme in both cell types. Clones with the highest expression produced greater than 100-fold (COX1) and 50-fold (COX2) more PGE2 than wildtype cells. In 10T1/2 clones, COX2 activities peaked within 1 or 2 days after seeding and declined as the cells approached confluency whereas COX1 activities showed less density-dependent activity. In high expressing AS52 clones, both COX1 and COX2 activities remained elevated at high cell densities. High levels of COX1 and COX2 were confirmed by Western and Northern analyses. These cell lines can be useful for study and comparison of biological effects (proliferation, transformation mutagenesis, etc.) caused by high expression of each enzyme. Additionally, they would be valuable in screening for nonsteroidal anti-inflammatory drugs (NSAIDS) which show isoenzyme specificity.

- T-1003** *In Vitro* Fertilization Enhances The Sensitivity And Utility Of The FETAX Assay. R. VARNOLD, E. Elmore<sup>1</sup>, and L.D. Smith. Developmental Biology Center, University of California, Irvine, Irvine, CA 92717; and <sup>1</sup>National Institute for the Advancement of In Vitro Sciences, Irvine, CA 92715.

The feasibility of adapting the FETAX (Frog Embryonic Teratogenesis Assay-Xenopus) assay to use *in vitro* fertilization was evaluated. The current FETAX assay as outlined in the ASTM guidelines consists of treating stage 8 to stage 11 embryos with decreasing concentrations of the test compound and monitoring for lethality and malformation of the embryos. 6-Aminonictinamide and 13-*cis*-retinoic acid, chemicals with known effects in the FETAX standard assay, were used in this assessment. Eggs fertilized *in vitro* were treated at the 4 cell stage and the development monitored through the same developmental stages used in the standard assay. The data was compared to the data obtained with embryos exposed as per the protocol in the ASTM guidelines. The preliminary data indicate that marked increases in sensitivity are possible with the new procedures. Both the LC50 (the concentration that is lethal to 50% of the embryos) and EC50 (the concentration that is effective in inducing birth defects) values are lower than previously reported suggesting that the assay is more sensitive to the test agents. In addition, two new morphological endpoints, gill and kidney malformation, that have not been previously reported with the assay were also observed. *In vitro* fertilization eliminates bias due to embryo selection, allows assessment of reproductive success, allows exposure to begin at stage 3 (4 cells) vs. stages 8 to stage 11 in the standard assay, and increases the sensitivity of the assay. Supported by the Johnson's Wax Fund.

**T-1004** Initial Characterization Of A New Model For Ocular Irritancy Testing. H.A. Ricker, P.J. Neal, J. Kubilus, and M. KLAUSNER. MatTek Corp., 200 Homer Avenue, Ashland, MA 01721.

A three-dimensional, tissue culture construct, EpiDerm-SCA, based on neonatal, foreskin-derived normal human epidermal keratinocytes (NHEK) has recently been developed by MatTek Corporation. Cultured using serum-free media in easily handled Millicell® CM (Millipore Corp.) cell culture inserts, the model allows application of liquid and solid test materials (potential ocular irritants) directly to the apical surface of these stratified, but non-cornified cultures. Initial toxicological testing of this model using the MTT assay with a graded series of shampoos gave reproducible results which correlated nicely with Draize ocular irritancy scores. In an initial study using a group of 8 final formulation shampoos dosed at 4 concentrations in triplicate wells (N=3), the average coefficient of variation was 8.1%. In addition, the EC-50 values (effective concentration of test material which reduces the MTT response to 50% of control cultures) when compared to non-washed Draize eye scores, had a correlation coefficient, *r*, of 0.71. Hence, it is anticipated that the model will have broad applications for non-animal eye irritancy testing.

**T-1005** Cellular Differentiation Is Enhanced In A Proliferating Culture Of Epidermal Keratinocytes Exposed To Bis-( $\alpha$ -chloroethyl)-Sulfide (BCES). L. BERNSTAM, A. Kottlyar, F.L. Vaughan, and I.A. Bernstein. The University of Michigan, Ann Arbor, MI 48109-2029.

Repression of DNA replication and mitosis is associated with exposure to BCES in submerged monolayer cultures of proliferating cutaneous keratinocytes derived from the newborn rat. Dose-responsively, the proliferative fraction of cells is decreased and a population of abnormally large cells, which do not replicate their DNA, appears in the culture. Study of the mechanism of this phenomenon has revealed the presence of a protein in the exposed culture that is found only in the cornified layer of the normal epidermis. A monoclonal antibody (7B4) which binds to this protein and which, by indirect immunofluorescence assay, stains only the cornified layer of the epidermis in the skin of the newborn rat, has been employed to study this response. Monolayer cultures were exposed to 10  $\mu$ M BCES for 30 min at 35°C. At 24 h post-exposure, cultures were treated with 7B4, an antibody generated by the hybridoma technique using murine cells, and the binding was visualized by application of anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate. More than 95% of the cells in exposed cultures showed fluorescence when viewed microscopically. In control cultures, less than 5% of the cells were stained. This observation supports the hypothesis that BCES switches cellular activity from proliferation to differentiation and provides a possible explanation for the observed decrease in DNA synthesis and mitosis in exposed cultures. (Supported by USAMRICD, DAMD17-90-C-0031.)

**T-1006** Appearance Of Interleukin 1 $\alpha$  (IL-1 $\alpha$ ) Can Be Used To Assess Cytotoxic Damage In Cultured Human Keratinocytes Exposed To Bis-( $\beta$ -chloroethyl)sulfide (BCES). Y. PU, P. Lin, F.L. Vaughan, and I.A. Bernstein. The University of Michigan, Ann Arbor, MI 48109-2029.

The utility of an increase in the level of IL-1 $\alpha$  as an indicator of cytotoxicity from BCES was evaluated in submerged monolayer cultures of human cutaneous keratinocytes. Two-day-old cultures were exposed to 1-100  $\mu$ M BCES at 35°C for 30 min. The amounts of IL-1 $\alpha$  in the medium and in the cells at 24, 48, and 72 h post-exposure were measured immunologically with an ELISA using antibody conjugated with peroxidase for visualization. Cell viability was measured concomitantly using the trypan blue dye-exclusion technique. The degree of interstrand cross-linking in the DNA of cells was determined by measuring the fluorescence resulting from the intercalation of ethidium bromide into double-stranded molecules that remained in heat-denatured DNA after exposure to BCES. Dose-responsive increases in the levels of IL-1 $\alpha$  in cells and in medium plus a decrease in the percentage of viable cells were observed. Comparison of the data showed a high correlation between the dose-responsive increase in the levels of IL-1 $\alpha$  in the medium and in the cells, and the dose-responsive decrease in the fraction of viable cells in the exposed cultures (e.g., *r* = -0.930 and -0.950, *p*<0.05, respectively) at 72 h post-exposure. The dose-responsive increase in the interstrand cross-linking found in the DNA of cells immediately after exposure to BCES, which correlated with loss of cell viability (*r* = -0.993, *p*<0.01), also showed a good correlation with the increases in IL-1 $\alpha$  observed in the medium (*r* = 0.982, *p*<0.05) and in the cells (*r* = 0.995, *p*<0.01) at 72 h post-exposure. These preliminary data suggest that the appearance of IL-1 $\alpha$  can be used to quantify the cytotoxicity resulting from BCES-mediated damage to cellular DNA. (Supported by the USAMRICD, DAMD17-90-C-0031.)

**T-1007** Assay Of Cytotoxicity Of Antimicrobial Agents By Correlation Of Keratinocyte Numbers With Optical Density Of Crystal Violet. S.T. BOYCE, C.Q. Sheeler, and I.A. Holder. Department of Surgery, University of Cincinnati and Shriners Burns Institute, Cincinnati, OH 45267-0558.

Cultured skin substitutes have become an alternative therapy for wound closure, but they are subject to microbial contamination and cytotoxic destruction by potent antimicrobial agents. For more rapid assay of cell proliferation, optical density of extracted crystal violet was correlated with cell numbers as a function of inoculation density (*r*<sup>2</sup> = 0.993), and of incubation time (*r*<sup>2</sup> = 0.999). Human keratinocytes were inoculated at 4 x 10<sup>3</sup> cells/well into 24-well plates, and incubated 3 days in modified MCDB 153 medium. On Day 4, baseline cell counts were performed, parallel cultures were fixed and stained with 0.1% (wt/vol) crystal violet, and combinations of antimicrobial agents were added to test cultures.

Test agents included constant conditions of polymyxin B, neomycin, mupirocin to which were added a quinolone (40 µg/mL), norfloxacin or ciprofloxacin; and an antimycotic, nystatin (100 U/mL) or amphotericin (0.25 µg/mL). On Day 6, parallel cultures were harvested and counted; or stained, extracted, and measured for optical density at 590 nm (OD<sub>590</sub>). Data are expressed as (mean ± SEM; n = 6) cell numbers and OD<sub>590</sub> and tested for significant (\*, p<0.05) cytotoxicity:

Condition	Cells/well (x10 <sup>-5</sup> )	OD <sub>590</sub>	p<0.05
Norf + Nys	0.67 ± 0.55	1.72 ± 0.01	*
Cipro + Nys	0.63 ± 0.74	1.32 ± 0.11	*
Norf + Amph	1.25 ± 0.06	2.19 ± 0.22	
Cipro + Amph	1.23 ± 0.08	2.75 ± 0.05	

The data show that colorimetric assay of crystal violet correlates reliably with cell numbers for assays of cell growth, and that this rapid assay can identify cytotoxic formulations of antimicrobials.

**T-1008** Coordinate Regulation Of Growth Factor Expression In Human Keratinocytes By 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. K.W. Gaido, L.S. Leonard, and S.C. MANESS. CIIT, 6 Davis Drive, Research Triangle Park, NC 27709.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a tumor promoter and carcinogen in rodents, is a potent modulator of epithelial cell growth and differentiation. In humans, the most well-characterized response to TCDD is chloracne, a skin disorder characterized by alterations in keratinocyte growth and differentiation. To understand the mechanism by which TCDD alters keratinocyte growth and differentiation, we investigated the cellular and molecular response of non-transformed human keratinocytes (NHK) to TCDD. Treatment with 10 nM TCDD enhanced NHK differentiation as determined by an increase in involucrin, a marker of keratinocyte differentiation. TCDD did not alter cell number of colony-forming efficiency, suggesting that TCDD enhanced the differentiation of cells already committed to terminal differentiation. TCDD altered the expression at both the mRNA and protein levels of a number of diverse growth factors including transforming growth factor-α (TGF-α), TGF-β<sub>2</sub>, urokinase plasminogen activator (u-PA), plasminogen activator inhibitor-2 (PAI-2), and interleukin-1β (IL-1β). TGF-α, u-PA, PAI-2, and IL-1β were induced 2- to 20-fold, while TGF-β<sub>2</sub> was reduced to 30% of control levels by TCDD. These results indicate that the alterations in keratinocyte growth and differentiation in response to TCDD are likely due to an effect of TCDD on normal growth-regulatory pathways.

**T-1009** Delayed Expression Of Cytotoxicity In Normal Human Epidermal Keratinocytes Treated With Shampoos. C. Juneja and C.W. Stott. Johnson & Johnson Consumer Products Worldwide, 199 Grandview Road, Skillman, NJ 08550.

Surfactants have the ability to induce phospholipid emulsification contributing to cellular damage, which can

result in cytotoxic processes. In order to reveal different mechanisms underlying cytotoxicity, we have investigated cellular protein contents, mitochondrial activity and membrane integrity in NHEK confluent cultures at 0, 4, and 24 hours using Coomassie Blue bioassay, MTS, and LDH leakage. Confluent cultures of normal human epidermal keratinocytes (NHEK) showed loss of mitochondrial activity, compromised membrane integrity and reduction in cellular protein immediately after treatment with shampoo formulations. However, no further loss of membrane integrity or decline in mitochondrial fraction occurred during the posttreatment period of up to 24 hours, following treatment. This indicates that the surviving cells were still viable and stable, but there is no indication of recovery or cell growth during this period. A continual decrease in attached cell protein over 24 hours, when taken by itself, would appear to be an expression of delayed cytotoxicity. When these other indicators of viability and membrane integrity are considered, it is evident that the action of the shampoos on the cells was immediate, resulting in metabolic death and lysis of the plasma membrane. The delay in detachment of all the dead cells or cell debris could best be detected at the 24 hour time point. In this model metabolic death is an early event whereas necrotic death is best measured at a much later time. The MTPA (Modified Total Protein Assay) is an assay of necrotic death.

**T-1010** Effect Of cAMP And Magnesium On Four Biochemical Markers Of Carcinogenesis. S. SHARMA, S. Zhu, and V.E. Steele\*. ManTech Environmental Technology Inc., Research Triangle Park, NC 27709 and \*NCI, Bethesda, MD 20892.

Cell proliferation plays an important role in carcinogenesis. Cyclic AMP and magnesium, the inhibitors for cell proliferation and growth, have been shown to be anticarcinogenic in animal models. Three cAMP analogs (8-bromo-cAMP, 8-chloro-cAMP and N<sup>6</sup>, O<sup>2</sup>-dibutyryl-cAMP) and three Mg<sup>++</sup> compounds (magnesium chloride, magnesium carbonate, magnesium hydroxide) were examined for their effects on four biochemical markers of carcinogenesis, which included the induction of reduced glutathione (GSH) in rat liver cells (BRL 3A), the inhibition of TPA-induced ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells (2C5), the inhibition of B(a)P activation and DNA binding in human bronchial epithelial cells (BEAS-2B), and the inhibition of carcinogen-induced poly(ADP)ribose-polymerase (PADPR) in human foreskin fibroblasts (HFF). Cells incubated for 18-24 hours were exposed to 5 log doses of the test agents (0.0001 mM-1.0 mM) for 5-24 hours, and assayed for various marker effects. Results indicated that all 6 compounds effectively induced the GSH level by up to 35%, significantly inhibited TPA-induced ODC activity by 40-100%, reduced the BaP activation and DNA binding by 15-65%, and suppressed the carcinogen-induced PADPR activity by 20-95%. The results suggest that the anticarcinogenic effect of these cell proliferation regulators may be achieved by a broad range of mechanisms.

- T-1011** In Vitro Chemosensitivity Assays Of Fresh Human Tumors: Culture In Liquid Versus Soft Agar Medium. A. Leyva, L.A. WETMORE, C. Zalles, and A. Freeman. The Children's Mercy Hospital, Kansas City, MO 64108.

Clonogenic assays used to characterize the tumor chemosensitivity of individual patients provide for substrate independent growth of tumor cells but not normal cells in semi-solid medium. However, technical problems include labor intensiveness and low evaluability due to low clonogenicity of tumor tissue. Alternative assays based on cell survival during short-term culture in liquid medium have led to improved handling and evaluability; but, potential problems with selectivity for tumor cells and relevance of drug doses have not been addressed. We have examined 128 surgical specimens from patients with various solid tumors for chemosensitivity using short-term (3-day) culture, determining cell survival by MTT tetrazolium dye reduction. Tumor cells were plated at 100,000/200  $\mu$ l in liquid or 10,000/ml in agar and then exposed continuously to drugs at multiple doses. 80% of tumors cultured in liquid had MTT absorbance >0.2 which provided good evaluability (majority >0.5). 70% of those tumors grown in agar had >20 MTT reactive cells, with a median of 180 or about 2% viable tumor cells. Nearly all specimens showed a very low growth fraction based on Ki-67 immunostaining. Specimens with a high proportion of normal cells as determined by H&E staining were evaluable with liquid but not agar culture. Viable tumor cells in agar were more sensitive to several anticancer agents at more clinically achievable doses. Using a cell survival assay in agar medium may provide a more clinically relevant model for in vitro drug testing of fresh human tumors.

- T-1012** Primary RTE Foci Inhibition Assay: Effects Of Vitamin A Derivatives As Potential Chemopreventive Agents. B.P. WILKINSON, S. Sharma, J. Arnold<sup>1</sup>, and V.E. Steele<sup>2</sup>. ManTech Environmental Technology Inc., P.O. Box 12313, Research Triangle Park, NC 27709; <sup>1</sup>University of North Carolina, Department of Pathology, Chapel Hill, NC 27599; <sup>2</sup>NCI, Bethesda, MD 20892.

A series of retinoids and its precursors were evaluated for their effectiveness as potential inhibitors of benzo(a)pyrene(B[a]P)-induced morphological transformation in primary rat tracheal epithelial (RTE) cells. RTE cells were isolated and exposed to B[a]P alone and in combination with various derivatives of nontoxic doses of vitamin A (natural or synthetic compounds) for 30 days. At the end of 30 days in culture the number of transformed foci was scored and the percentage of inhibition in chemopreventive agent treatments was quantitated. In the RTE assay, 73% of the retinoids tested were positive, especially the lower concentrations being most effective in inhibiting foci formation. Test results further indicate that most of the retinoids tested were 100% inhibitory, whereas carotenes were 50-60% inhibitory with the exception of  $\alpha$ -carotene, showing an

inhibition of 89-100%. Both forms of vitamin A have been shown to inhibit carcinogenesis by promoting cell differentiation and a variety of other mechanisms including inhibition of oxidation and mutation, inactivation of free radicals and enhancing immune functions. A number of natural and synthetic retinoids are presently undergoing clinical trials as chemopreventive agents. (Supported by NCI Contract #N01-CN-55503-03, -05, #N01-CN-95172-02, -06, and NCI-CN-25466-01.)

- T-1013** Screening Of Eighty-Seven Compounds For The Inhibitory Effect On Carcinogen-Induced Poly(ADP)ribose Polymerase Activity In Human Foreskin Fibroblasts. G. WYATT, S. Zhu, S. Sharma, and V.E. Steele\*. ManTech Environmental Technology Inc., Research Triangle Park, NC 27709, and \*NCI, Bethesda, MD 20892.

Poly(ADP)ribosylation is involved in the processes of DNA repair, sister chromatid exchange and cell differentiation, which may contribute to carcinogenesis, and the inhibition of PADPR polymerase activity may serve as a screening system for potential chemopreventive agents. 87 compounds were examined for their inhibitory effect on a direct carcinogen propane sultone-induced PADPR activity in human foreskin fibroblasts. Cells were plated for 18-24 hours and treated for another 24 hours with 5 log doses of the test agents (0.0001 - 1.0 mM) and/or propane sultone. Cell free extract were prepared and assayed for PADPR activity using <sup>32</sup>P-NAD as substrate in the presence of histone and activated DNA. 52/87 compounds (60%) are classified as strong inhibitors, based on >60% inhibition in 3 doses, >20% inhibition in all doses, or dose-dependent inhibition. Cyclic AMP analogs (3/3), magnesium compounds (3/3) sulfurs (8/8), phenols (5/5), and terpenes (8/10) show preferential inhibitory activity in this assay system. The application of this assay to the screening of potential chemopreventive agents will be discussed.

- T-1014** Screening Of Eighty-Five Compounds Using Inhibition Of Benzo(a)pyrene-DNA Binding In BEAS-2B Cells As A Biochemical Marker For Carcinogenesis. S. ZHU, E. Korytynski, S. Sharma, and V.E. Steele\*. ManTech Environmental Technology Inc., Research Triangle Park, NC 27709, and \*NCI, Bethesda, MD 20892.

The activation of polycyclic aromatic hydrocarbons and the binding of the activated products to macromolecules seems to be correlated to carcinogenic process. Eighty-five compounds were screened as chemopreventive agents in a benzo(a)pyrene activation and DNA binding assay in human bronchial epithelial cells (BEAS-2B). The cells were cultured for 18-24 hours before a 6-hour exposure to the test agents (0.0001 mM - 1.0 mM). Two hours after the initiation of chemical exposure, cultures were treated with 1  $\mu$ M <sup>3</sup>H-BaP for 4 hours. The cells were then processed for DNA isolation and precipitation. Aliquots were used for DNA content and radioactive measurements. Forty-seven compounds (58%) showed strong inhibitory effect on B(a)P-activation and DNA binding in the test system, as indicated by

>20% inhibition in 3 doses, or >40% inhibition in 2 doses. High percentage of certain classes of chemicals shows positive inhibitory effect in this assay, which includes cAMP analogs (3/3), magnesium compounds (3/3), sulfurs (7/7), phenols (4/4), and terpenes (8/9). The significance of this assay in screening of chemopreventive agents is also discussed.

**T-1015** Evaluation Of Involucrin Expression In Human Epithelial Cells For Possible Utility For Screening Cancer Chemopreventive Agents *In Vitro*. E. ELMORE<sup>1,3</sup>, C. Sun<sup>2</sup>, J.A. Buckmeier<sup>2</sup>, and J.L. Redpath<sup>2</sup>. <sup>1</sup>National Institute for the Advancement of In Vitro Sciences, Irvine, CA 92715; <sup>2</sup>Department of Radiation Oncology, <sup>3</sup>Division of Hematology Oncology, University of California Irvine, Irvine, CA 92717.

Cancer chemopreventive agents can inhibit propane sulfone enhanced growth of primary human epithelial cells. One possible mechanism for this inhibition may be induced differentiation. Involucrin expression, a cellular differentiation marker, following co-exposure to propane sulfone and potential chemopreventive agents was used to test this hypothesis. Cells were cultured in medium containing propane sulfone both with and without chemopreventive agents. After four subcultures, the cells were seeded in 96 well dishes for the assessment of involucrin expression. After 7 days of growth, the cells were immunoperoxidase stained using a polyclonal antibody to involucrin. Cells in a parallel 96 well plate were stained with methylene blue to assess growth. The results indicate that growth inhibition is associated with involucrin induction and suggest that involucrin expression may be a useful biomarker for chemopreventive agent screening in human epithelial cells.

**T-1016** *In Vitro* Expression Of Heat Shock Proteins Associated With Hyperthermia And Ionizing Radiation Exposure In Human B-Cells. S.L. SCHNEIDER, S.A. Fuqua, M. Szekeresova, and M.L. Meltz. The University of Texas Health Science Center, Departments of Radiation Oncology and Medical Oncology, San Antonio, Texas 78284.

A universally observed response of organisms to heat is the induction of heat shock or "stress" proteins (HSPs). Cells exposed to various forms of environmental stress respond by increasing the synthesis of highly conserved HSPs. The down-regulation of B-cell proliferation and growth is suggested to be directly involved with the expression of a low molecular weight HSP. The normal, human, Epstein Barr Virus-transformed, lymphoblastoid cell line 244B, with a phenotype of activated peripheral blood cells, was used as an *in vitro* B-cell model to study the expression of HSPs p70, p27, and heat shock transcription factor (HSTF) following 42° C hyperthermia and 50-1000cGy standard dose rate ionizing radiation (SDRR) before and after hyperthermia exposure. Maximal expression of HSPs was observed following 42° C/1.5 h. Inhibition of induced HSPs was observed when 250cGy SDRR exposure immediately followed 42° C treatment. The gel retardation HSTF assay indicated that this inhibition of p70 and p27 was

not at the transcription level. Understanding the general controls of protein initiation and inhibition at the molecular level may help define the effector mechanism which regulates cellular proliferation rate and "programmed cell death" following ionizing radiation exposure in peripheral blood B-cells. (This study was funded by grant AFOSR-91-0206-A, Armstrong Laboratory, Brooks Air Force Base, TX, and the San Antonio Cancer Research Institute, San Antonio, TX.)

**T-1017** Development Of Human B-Lymphocyte Cultures Secreting Antibodies To *Botulinum* Neurotoxin. C. WEBB<sup>1</sup>, J. Testa<sup>2</sup>, J. Middlebrook<sup>2</sup>, and B. Butman<sup>1</sup>. <sup>1</sup>Organon Teknika/Biotechnology Research Institute, Rockville, MD 20850; <sup>2</sup>USAMRIID, Frederick, MD 21701.

Human B-cell cultures secreting antibodies against *Clostridium botulinum* neurotoxin were developed using a novel transformation procedure. Peripheral blood lymphocytes (PBL) from toxoid-immunized donors were used as the source of B-lymphocytes for *in vitro* toxin stimulation and immortalization. B-cells were enriched by T-cell depletion, further stimulated *in vitro* with native toxin and then immortalized using Epstein-Barr virus transformation in the presence of a mixture of cytokines and immunomodulators (IL-4, IL-6, and 8-mercaptopguanosine). Transformed B-cells were cultured for an appropriate time and the supernatants were analyzed for the presence of antigen-specific immunoglobulins. The primary screening consisted of an indirect ELISA utilizing toxin bound to microtitration plates. From three transformations, a total of 197/1248 primary human B-cell cultures were developed that produce toxin-reactive antibodies against *Clostridium botulinum*. Toxin-reactive culture supernatants were also tested for their ability to neutralize toxin using a mouse model. None of the cultures were found to produce neutralizing antibodies although toxoid-derived polyclonal antibodies (human and equine) did neutralize toxin. The absence of neutralizing capability by the culture supernatants could be due to the failure to isolate human B-lymphocytes that recognize a neutralizing epitope on the toxin. Alternatively, antibody-mediated neutralization of *Clostridium botulinum* neurotoxin may require binding at more than one epitope.

**T-1018** Differential Mitomycin C-Induced Cell Cycle Inhibition and Apoptosis In A B And T Lymphoblastoid In Vitro System. S. BLOOM, M. Potchinsky, and D. Muscarella. Department of Avian & Aquatic Animal Medicine, Cornell University, Ithaca, NY 14853.

Certain drugs cause selective toxicity towards B lymphocytes as compared to T lymphocytes. In the cases of cyclophosphamide and mitomycin C (MMC), the selective eradication of B cells from immune tissues depends mainly on mechanisms operative within B and T lymphocytes. We have investigated mechanisms of MMC-induced selective toxicity in model avian B and T lymphoblastoid cell lines. The B- (BLC) and T-lineage (TLC) lymphoid cell lines, DT40 and CU159, respec-

tively, were exposed to graded dosages of MMC for one hour, and allowed to recover in drug-free culture medium. Similar levels of MMC-induced DNA-DNA cross-linking were detected in the ribosomal RNA gene clusters of the BLC and the TLC. Progression of cells over multiple cell cycles was inhibited in the BLC (0.01 - 0.10 µg/ml) but not in the TLC. Selective blockage in the cell cycle, extending from late S into G<sub>2</sub> and M phases, was observed in the BLC only. Partial blockage of TLC cell cycle progression was achieved at a 40-fold higher dosage of MMC. Growth of B-lineage cells was dramatically inhibited and cells displayed a phenotype indicative of an apoptotic mode of cell death. In contrast, induction of apoptosis was not as extensive and was delayed in T-lineage cells. These results demonstrate selective MMC-induced inhibition of cell cycle progression and engagement in apoptosis in B-lineage cells. This appears to involve the differential capacity of B- and T-lineage cells to replicate with damaged DNA and to enter a cell death cascade.

**T-1019** *In Vitro* Effect Of Organochlorine And Organophosphate Pesticides On Human Peripheral Blood Mononuclear Cells (HPBMNC). V. RAMA KRISHNA and P.R. Rao. Immunobiology Lab, Department of Zoology, Osmania University, Hyderabad-500 007, India.

Pesticides constitute an important group of chemicals that affect the immune system. To evaluate the direct action of organochlorine and organophosphate pesticides, five each of structurally different organochlorine (Aldrin, Butachlore, Endosulfan, DDT, HCH) and organophosphate (Malathion, Parathion, Quinolphos, Monochrotophos, Phaspomidon) were selected and studied for *in vitro* effect of these pesticides on HPBMNC. HPBMNC were exposed to two concentrations (low concentration at 10<sup>-15</sup> g to 10<sup>-17</sup> g/ml and high concentration at 10<sup>-6</sup> g/ml) of each pesticide and their proliferative responses to con A and Interleukin-2 (IL-2) production were studied. Along with these studies we also analyzed various CD4+ and CD8+ cell populations in pesticide pretreated HPBMNC. Both these groups of pesticides suppressed lymphoproliferative responses to con A IL-2 production at higher concentration (10<sup>-6</sup> g). But at low concentration only organochlorines showed significant enhancement on lymphoproliferation and IL-2 production. Our FACS analysis study showed depletion of CD4+ cells, and no change in CD8+ cells was observed at higher concentration. But at low concentration no change in CD4+ and CD8+ subsets was observed. These results indicate that these pesticides are directly immunosuppressive to isolated cells at higher concentrations without the interference of other organ systems.

**T-1020** Quality Control Of L929 Cells For Use In In Vitro Cytotoxicity Methods: A Test Battery To Reveal Passage-Dependent Alterations In Cellular Responses. J.F. HAMBERGER, C.J. Peters, and C.B. Jessee. Bausch & Lomb, Rochester, NY 14692.

The mouse L929 fibroblast continuous cell line is widely used for *in vitro* cytotoxicity testing, and is speci-

fied in U.S. Pharmacopeia XXII for In Vitro Biological Reactivity Tests for evaluating the cytotoxicity of plastic extractables. Such *in vitro* tests using L929 cells are often used to pre-screen samples tested *in vivo* and are also used as Quality Control tests for lot release of raw materials and finished products. A visual reduction in metabolic acidification of the Phenol Red medium with increasing passage time caused us to further assess the potential effects of passage number by a battery of cytotoxicity indicators. Both untreated cells and cells exposed to graded doses of the non-ionic surfactant Tyloxapol were evaluated using four indicator assays: 1) Neutral Red Dye Release, which measures plasma/lysosomal membrane integrity; 2) alamarBlue Dye Reduction, which measures metabolic reduction by enzymes such as LDH of a tetrazolium salt; 3) Glucose Consumption, which measures glucose utilization by respiring cells; and 4) Propidium Iodide Dye Viability, which measures nuclear exclusion of dyes to quantitate cell population viability. Results indicate that rate and extent of Neutral Red Dye Release from early and late passages differs. Measurement of cell recovery following Tyloxapol insult by alamarBlue Dye Reduction indicates that early and late passage cells respond differently as well. Glucose Consumption assays also indicate altered metabolic potential of late passage cells. Differences in rates and extents of viability reduction in response to insult were also observed by nuclear dye exclusion. These results demonstrate the importance of implementing appropriate Quality Control standards in the monitoring of cell lines used for *in vitro* cytotoxicity testing. Furthermore, the different response profile of different cell line passage numbers underscores the need to calibrate and validate appropriate conditions for the use of a cell line for specific assay methods.

**T-1021** Induction Of 70,000-Da Heat Shock Protein In HeLa Cells By Mercury. HIROSHI OSHIMA<sup>1</sup>, Takumi Hatayama<sup>2</sup>, and Masaaki Nakamura<sup>1</sup>. <sup>1</sup>Department of Biomaterials, Osaka Dental University, 5-31 Otemae 1-chome, Chuo-ku, Osaka, 540, Japan; <sup>2</sup>Department of Biochemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto, 607, Japan.

To clarify the biochemical changes of the cells caused by biomaterials, induction of heat shock proteins (HSPs) by mercury ion, which is known to be cytotoxic, and is one of the main components of dental restoratives, was examined. Exponentially growing HeLa 229 cells were incubated in Eagle's MEM supplemented with 10% calf serum containing various concentrations of mercuric chloride for various times. To analyze the synthesis of HSPs, the cells were incubated in 1.0 ml of methionine-free medium containing 10-20 µCi/ml <sup>35</sup>S-methionine in the presence of mercuric chloride. After the labeling, the radioactivity incorporated into the protein was assayed as the counts precipitated with hot trichloroacetic acid, and the labeled proteins were separated by SDS-PAGE. Synthesis of HSPs was evaluated by densitometry of the autoradiograms of the gels. A cytotoxicity test with neutral red uptake was concomitantly carried out. Incubation of the cells in the medium

containing 1.25 to 40  $\mu\text{M}$  mercuric chloride markedly increased the synthesis of 70,000-Da HSP (hsp 70). At 20 and 40  $\mu\text{M}$  mercuric chloride, the synthesis of hsp 70 increased from 1 h after exposure, reached maximum at 3 h, and gradually decreased thereafter. The present findings suggest that synthesis of hsp 70 represents the cellular changes caused by noxious substances. Further studies are needed to elucidate the physiological role of the proteins in biomaterial testing.

**T-1022** Factors Influencing Metal Toxicity In Continuous Cell Lines. JANET T. JONES, Kevin K. Divine, Marc Oshiro, Dean Carter, and Hugh E. Laird II. Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ 85721.

Continuous cell lines are an important tool in the study of mechanisms of toxicity of environmental chemicals. Study of these mechanisms requires culture of such cells for long enough times that pre-lethal and sub-lethal events can be seen. The cells must be tested in media that do not adversely affect cell viability. Toxicity of salts of three metals, mercury, cadmium, and zinc, has been studied in cell lines derived from liver, kidney, and prostate, organs affected by *in vivo* exposure to these metals. We have shown that: a) use of fetal calf serum in assays has a protective effect on toxicity that varies with cell type and metal; b) the culture medium in which cells are grown and tested alters their sensitivity to metal toxicity; c) cell lines of different tissue origin vary in their sensitivity to metals, prostate cells being less sensitive to zinc and cadmium than liver and kidney cells; d) incubation of liver and kidney cells for 46 h rather than 23 h in metal salts does not increase toxicity. *In vivo*, metals are bound to endogenous ligands (e.g. albumin, glutathione, metallothionein). Similarly, metals form complexes with cellular and medium components in cell culture. To study the effects of specific metal complexes on toxicity, metal-albumin and metal-glutathione complexes have been made and characterized. The toxicity of these complexes has been found to vary with metal, ligand, and cell type. Mercury-glutathione is less toxic than  $\text{HgCl}_2$  for all three cell lines. The effect is similar if glutathione and  $\text{HgCl}_2$  are added to the medium in an uncomplexed state. A cadmium-albumin conjugate was less toxic than  $\text{CdCl}_2$  in all three cell lines, as were cadmium and albumin added in unconjugated form. The addition of albumin did not protect against mercury toxicity. (Supported by NIEHS grant #ES04940).

**T-1023** Interaction Between Ca, Cd, And Zn And The Actin Filaments In MDCK Cells. J.W. MILLS, G. Church, and J. LaCroix. Department of Biology, Clarkson University, Potsdam, NY 13699-5805.

Exposure of MDCK cells to 10  $\mu\text{M}$  cadmium (Cd) or zinc (Zn) leads to an increase in the amount of F-actin and a change in distribution of actin filaments within the cell (Mills et al., *Tox. Appl. Pharm.* 116:92, 1992). Since these two divalent cations are known to interact with calcium (Ca) we examined the effect of changes in extracellular Ca. Raising the Ca to 5 mM completely

blocks the Cd effect while having no effect on Zn. Reducing Ca below normal levels (1 mM) accelerates the effect of both metals. This indicates one potential site of interaction between these cations may be via entry at the Ca transport sites in the cell membrane. We investigated whether treatment of cells with different Ca channel blockers also altered the response to Cd or Zn. Verapamil, nifedipine and diltiazem, added either simultaneously with or prior to the addition of the metals, failed to block the effect of either Cd or Zn. In order to determine if the individual metal effects were related to changes in Ca uptake we examined  $^{45}\text{Ca}$  entry into suspended cells. We found that the Ca uptake at 30 sec and 3 min was not altered after exposure to as much as 100  $\mu\text{M}$  Cd or Zn. Analysis of the F-actin content in the suspended cells demonstrated that the Cd effect still persisted as there was a 48% increase in Cd-treated cells as compared to controls. No significant effect of Zn on F-actin content could be detected in suspended cells. We conclude that the competition between Ca, Cd, and Zn, as it relates to changes in actin filaments, does not occur at the entry step for these cations but rather is at an intracellular site. (Supported by NIH - ES06392).

**T-1024** Induction of NOS Gene Expression by TPA in Primary Neonatal Rat Hepatocytes. U. ARMATO, M. Menegazzi, C. Guerriero, C. Cardinale, J. Wu, A. Carcereri de Prati, S. Mariotto, and H. Suzuki. Institutes of Anatomy & Histology and Biochemistry, University of Verona, I-37134, Italy.

Inducible nitric oxide synthase (iNOS) is induced by a variety of stimuli, such as bacterial endotoxins, interferon, or cytokines, in hepatocytes. However, the pathophysiological aim of this activation remains obscure, and the possible roles, if any, of iNOS in hepatocyte proliferation and in hepatocarcinogenesis wait to be assessed. To clarify these topics, we undertook the present work using primary cultures of Percoll-purified neonatal hepatocytes attached to polyethylene disks floating on the top of the medium and treated with the archetypal tumor promoter phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA;  $10^{-9}$  mol/L) [1]. Our findings show that TPA, dissolved in the MEM medium, was mitogenic for a significant fraction of the quiescent neonatal hepatocytes. In the same cells, TPA markedly induced, already within the first 30 min, the levels of the mRNA encoding for the iNOS. The mRNA levels peaked after 1 hour and thereafter declined. No change in iNOS mRNA levels occurred in the untreated, parallel liver cultures. These observations lend credence to the view that iNOS may mediate the mitogenic actions of nongenotoxic carcinogens in neonatal hepatocytes. Hence, iNOS might partake to the mechanisms underlying the promotion of liver chemical carcinogenesis.

[1] F. Romano, L. Menapace, U. Armato, *Carcinogenesis* 9:2147-2154 (1988). (Work supported by A.I.R.C. [Milan], M.U.R.S.T. [40%, 60%], and C.N.R.).

**T-1025** Immortalization Of Human Endothelial Cells By SV40 Virus: Possible Cooperative Effect Of Abnormal P53 Gene. D.R. UMBENHAUER, L. Toji, T.E. Johnson, R.B. Hill, and W.W. Nichols. Merck Research Laboratories, Department of Genetic and Cellular Toxicology, West Point, PA 19486 and Coriell Institute for Medical Research, Camden, NJ 08103.

SV40 virus is thought to exert its transforming effect through the binding of SV40 large T-antigen to growth regulatory proteins, including P53, thereby inactivating crucial growth controls. Human iliac artery endothelial cells were infected with SV40, resulting in morphologically transformed cells that grew more vigorously than the mock-infected culture and eventually entered a crisis period. An immortalized cell line, capable of forming tumors in nude mice, was obtained from the surviving cells of one infected culture. A detailed chromosome analysis was carried out on the pre- and post-crisis cells and on three solid tumors. Each of the transformed cultures contained numerous chromosomal abnormalities. With the progression from morphologically transformed to immortalized cells to solid tumors, the chromosomal markers became more consistent, reflecting a clonal selection process. All of the tumor cells had a deletion on chromosome 11 with the breakpoint at band p13, the site of the tumor suppressor gene involved in Wilms' tumor. Southern analysis using a WT-1 probe did not reveal any differences between the parent and the transformed cultures. Other common markers in the tumor cells include a 6p+(p25) and a deletion on chromosome 10 at p13. An abnormality of chromosome 17 was frequently seen at all stages of transformation, with most of the abnormalities localized to band p13, the site of p53 tumor suppressor gene. Southern blots showed an abnormal p53 allele in the transformed cells and in tumor DNA, but not the parental culture. This "mutant" allele was also amplified in one of the tumors. These results suggest that an abnormal p53 may confer a growth advantage, even in cells that already express T-antigen.

**T-1026** Cell Cycle-Dependent Modulation Of Protein Kinase C (PKC)-Related Signal Transduction By 2,3,7,8-Tetrachloro-Dibenzo-P-Dioxin (TCDD). T.J. WEBER and K.S. Ramos. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843.

Recent studies in this laboratory have suggested that the modulation of PKC activity by TCDD, a widespread environmental carcinogen, is influenced by cell cycle progression. To further test this hypothesis, experiments were conducted to examine the effects of TCDD on PKC activity, AP1 binding to a synthetic phorbol ester (TPA) responsive element (TRE), and DNA synthesis in subcultured vascular smooth muscle cells (SMCs). These cells allow for synchronization of cell cycle entry and progression and thus afford the opportunity to study growth-related events. C-kinase activity was modulated in a cell cycle-related manner where decreased activity was observed in the cytosolic and particulate fractions of SMCs treated with 10 nM TCDD during G<sub>0</sub> as well as 8 h immediately following

serum-induced cell cycle progression, increased activity was observed in both fractions at 8-16 h after serum stimulation, and no changes in activity occurred at 16-24 h. Pretreatment of SMCs with cycloheximide prior to TCDD exposure during the 8-16 h period also resulted in increased PKC activity suggesting that modulation was not related to de novo protein synthesis. TCDD was unable to induce AP1 binding to a TRE in quiescent SMCs and reduced serum-stimulated AP-1 activity. A reduction of DNA synthesis was observed only when SMCs were treated with TCDD during the G<sub>0</sub>-G<sub>1</sub> period of the cell cycle, but not 8-24 h after serum stimulation. These data demonstrate that discrepancies noted in the literature for the requirement of serum in the toxic responses induced by TCDD may be cell cycle-related.

**T-1027** In Vitro Cytotoxicity Testing: 72-Hour Studies With Cultured Lung Cells. F.A. BARILE and D. Alexander. Department of Natural Sciences, City University of New York at York College, Jamaica, NY 11451.

This study was designed to evaluate the potential of an *in vitro* cell culture method for its ability to determine cytotoxicity and to compare the cytotoxic concentrations with established human and animal toxicity data. Rat lung epithelial cells (L2) and human fetal lung fibroblasts (HFL1) were incubated in the absence or presence of increasing concentrations of test chemicals for 72-hours, and cell proliferation was used as a marker for toxicity. Inhibitory concentrations were extrapolated from concentration-effect curves after linear regression analysis. Comparison of the cytotoxicity data, from testing 20 chemicals, with rodent and human lethal concentrations suggests that the experimental IC50 values are as accurate predictors of human toxicity as equivalent toxic blood concentrations derived from rodent LD50s. In addition, 72-hour growth studies were more sensitive to cytotoxicity than previously performed 24-hour protein synthesis experiments. It is anticipated that these procedures, together with a related battery of tests, may supplement or replace currently used animal protocols to screen chemicals for human risk assessment. (Supported in part by NIGMS GM08153).

**T-1028** Comparisons Of The Toxicity Of 30 Chemicals As Measured By 68 Different In Vitro Toxicity Tests. C. CLEMEDSON\*, E. Abdulla, F.A. Barile, C. Chesné, R. Clothier, M. Cottin, R. Curren, P. Dierickx, M. Ferro, G. Fiskesjö, L. Garza-Ocanas, M.J. Gómez-Lechón, M. Gulden, B. Isomaa, A. Kahru, R.B. Kemp, G. Kerszman, U. Kristen, M. Kunimoto, S. Kärenlampi, K. Lavrijsen, L. Lewan, T. Ohno, G. Persoone, R. Petterson, R. Roguet, L. Romert, T. Sawyer, H. Seibert, R. Shrivastava, M. Sjöström, N. Tanaka, F. Zucco, and B. Ekwall\*. Department Pharmaceutical Biosciences, Uppsala University, Box 594, S-75124 Uppsala, Sweden.

In the ongoing MEIC evaluation project, we have now tested the first 30 reference chemicals in 68 different *in vitro* toxicity tests, including 15 with human cell lines, 3 with human primary cultures, 18 with animal cell lines, 21 with animal primary cultures and 11

ecotoxicological tests. The results have been compared with use of linear regression and variance analysis. First, the similarity of all methods was determined. Principal component as well as pairwise variance analysis indicated an 80% similarity. Second, the influence of specific components of methods on the general variability of the data was studied: 1) Exposure time was found to increase toxicity for many chemicals; 2) Human cytotoxicity was well predicted by animal cytotoxicity tests (except for digoxin and malathion), and less well by ecotoxicological tests; 3) The few organotypic toxicity criteria used, e.g. contractility, gave different results compared with the basal cell function criteria; 4) Twelve comparisons of identical test systems using different cell types (including primary cultures) showed a similar toxicity regardless of cell type; and 5) Ten comparisons of test systems with identical cell types and exposure times, revealed a similar toxicity regardless of the endpoint used. Thus, factors 1-3 contributed to the 20% dissimilarity, while 4 and 5 must be the main factors explaining the remarkable similarity of the total results. The findings confirm earlier results (B. Ekwall et al., *In Vitro*, 28, 3, 1992, 135A), support the basal cytotoxicity concept, and will indeed facilitate in vitro toxicity testing.

**T-1029** H<sub>2</sub>O<sub>2</sub> Induced Perturbation Of Neuronal Physiology And Morphology. J.M. Ryan, P.J.S. SMITH and M. Tytell. NVPF, M.B.L., Woods Hole, MA 02543.

This research has been interested in developing an invertebrate model for the study of a free-radical attack on the normal physiological and morphological characteristics of neurons. We have been using the easily cultured, relatively large neurons from the opisthobranch mollusc, *Aplysia californica*. The Bag Cell neurons from these animals regenerate within 24 h when isolated in a simple solution of artificial seawater. We assume that the applied H<sub>2</sub>O<sub>2</sub> generates free-radicals via a Fenton reaction involving trace iron in the seawater.

We have measured the effect of a free-radical attack on the membrane potential, the trans-membrane potassium and calcium flux, axoplasmic transport and overall morphology. All are profoundly effected. Transmembrane potassium flux reflects the depolarization of the membrane perhaps resulting from the failure of the sodium/potassium pump. Growth cones rapidly bleb and axonal transport is stopped even at low H<sub>2</sub>O<sub>2</sub> concentrations ( $\approx 1 \mu\text{mol}\cdot\text{l}^{-1}$ ). Calcium fluxes change but so do soma morphologies and as yet we cannot be sure the two are not connected. Eventually, after 1-2 h at  $5\text{-}10 \mu\text{mol}\cdot\text{l}^{-1}$ , the cells lyse. All these effects are ameliorated by the presence of the known transition metal chelator and free-radical scavenger, albumin. Of interest is preliminary data which suggests that when cells are preincubated in, and washed clear of, heat shock protein 70 (Hsp70) the attack is significantly retarded with regard to the degeneration of axonal structure. Hsp70 is produced in conditions of stress as part of a cell's molecular defense. We are currently investigating the effects of this compound on the physiological parameters and axon transport.

**T-1030** Cellular Accumulation Of Carotenoids By HepG2 And Caco-2 Human Cell Lines. K.R. MARTIN, M.L. Failla, and \*J.C. Smith, Jr. University N. Carolina at Greensboro, NC 27412 and \*Beltsville Human Nutrition Center, USDA, Beltsville, MD 20705.

Epidemiologic studies support the hypothesis that dietary carotenoids may decrease the risk of disorders such as atherosclerosis, cataractogenesis, and neoplasia. One proposed mechanism by which carotenoids exert their protective influence involves quenching of free radicals that induce oxidative damage. We have initiated studies with Caco-2 and HepG2 human cell lines as models to investigate the putative cytoprotective influence of carotenoids. At confluency, Caco-2 differentiates into a polarized, enterocyte-like cell possessing a brush border surface and tight junctions between cells. HepG2, which is derived from a hepatocellular carcinoma, secretes plasma proteins and has an inducible cyt. P450 system. Representative carotenoids, viz. beta carotene (BC), lutein (LUT), and canthaxanthin (CXN), have been selected for study. The initial concern was the presentation of these lipophilic compounds in a bioavailable form. To this end, micelle preparations (50  $\mu\text{M}$ ) were prepared in complete cell culture media and passed through a 0.22  $\mu\text{M}$  filter to remove insoluble materials. Filtration efficiencies for all 3 carotenoids were routinely 70-80%. Carotenoid containing media were incubated at 37° C to assess chemical stability. Spectrophotometric analysis indicate 85-93% of carotenoids remained in solution after 3 days. Exposure of cells to BC, LUT, and CXN did not induce morphological changes. Levels of BC, LUT, and CXN in Caco-2 after 1 day were 2.9, 5.7, and 6.3  $\mu\text{g}/\text{mg}$  protein, respectively. HepG2 exhibited similar levels of accumulation. Extending the incubation period to 3 days resulted in 2-4 fold increase in cellular levels of carotenoids. Studies to assess potential protective effect of carotenoids against oxidative stresses are ongoing.

**T-1031** Primary Rat Hepatocyte Cultures Aid In The Chemical Identification Of Toxic Chaparral (*Larrea tridentata*) Fractions. D. PRITCHARD, W. Obermeyer\*, J. Bradlaw, W. Roth, T. Flynn, J. Yates, and S. Page\*. FDA, Division of Toxicological Research, Laurel, MD 20708. \*FDA, Division of Natural Products, Washington, DC 20204.

Leaves and twigs of chaparral (creosote bush, *Larrea tridentata*) have been used in herbal dietary supplements and teas. Cases of acute non-viral hepatitis have been linked to the consumption of certain chaparral products. To help identify the biologically active components, primary rat hepatocytes were exposed to chemical fractions of chaparral prepared by exhaustive extraction of leaves and twigs with four solvents of differing polarity: petroleum ether (PE), dichloromethane (DCM), methanol (MeOH), and water (H<sub>2</sub>O). Monolayer hepatocyte cultures were exposed to different levels of diluted extracts in culture medium for up to 48 h. Comparative cytotoxic measurements were made for exposed cultures based on the number of cells that remain attached

to the culture surface after 24 h and morphological changes after 48 h. The order of toxicity based on the lowest observed-effect level (LOEL) was MeOH > DCM > PE > H<sub>2</sub>O. The methanolic extract was subfractionated into a water-soluble fraction and a nonsoluble phenolic fraction. The LOEL for the phenolics was approximately 0.75 mg equivalents/mL of chaparral, while the LOEL for the water-soluble subfraction was approximately 10 mg equivalents/mL. Additionally, attached hepatocytes were exposed to different levels of chaparral tea diluted in medium. The LOEL, approximately 5 mg equivalents/mL, was attained only at highly concentrated levels of reconstituted freeze-dried tea. These preliminary findings will help to focus further studies to establish the hepatotoxic principles of chaparral.

**T-1032** In Vitro Assessment Of Fumonisin B<sub>1</sub> Toxicity Using Reaggregate Cultures Of Chick Embryo Neural Retina Cells (CERC). J. BRADLAW, D. Pritchard, T. Flynn, R. Eppley\*, and M. Stack\*. FDA, Division of Toxicological Research, Laurel, MD 20708; \*FDA, Division of Natural Products, Washington, DC 20204.

Fumonisin, mycotoxins produced by several *Fusarium* species, including *F. moniliforme*, have been reported to cause neuronal degeneration, liver and renal toxicity, cancer and other injury to animals. In addition, an association of fumonisin with severe adverse pregnancy outcomes has been suggested. Fumonisin B<sub>1</sub> (FB1) is the major fumonisin found in corn and corn products intended for human use. The CERC assay has broad utility as a preliminary screen for developmental toxicants. Early events of development are measured, including cell-cell interaction (cell reaggregation), growth and differentiation. Reaggregate cultures of Day 6.5 CERC were exposed to FB1 at graded dose levels, 0.1 to 10 mM, in serum free medium. At the end of a 24-h treatment period, the size distribution of the aggregates was measured. After a further culture period of 6 days in complete medium, a differentiation marker for Müller glial cells, glutamine synthetase activity (GSA), was determined. Growth was expressed as total protein per culture. FB1 was found to inhibit cell aggregation in a dose-dependent manner. The lowest observed effect level (LOEL) was 2.5 mM for inhibition of aggregation. When the aggregates were resized 6 days after the removal of FB1, they increased in size at all levels except at 10 mM. The LOEL for total protein was 5 mM. However, FB1 did not inhibit GSA. In contrast, positive controls 5'-bromodeoxyuridine at 25 μM and vinblastine at 11 nM inhibited GSA and aggregation, respectively. The presence of FB1 for 48 h during the aggregation period or during the cell proliferation and differentiation stages of development did not appreciably change the final outcome of the assay. FB1 affected two endpoints in the CERC assay, cell aggregation and total protein. This result is consistent with identification of FB1 as a potential developmental toxin.

**T-1033** In Vitro Cytotoxicity Of Trypanocides. R. KAMINSKY, C. Schmid, and R. Brun. Swiss Tropical Institute, CH 4002 Basel, Switzerland.

Searching for new antitrypanosomal compounds has been substantially accelerated by the development of in vitro screening assays (1). To obtain an indication of potential cytotoxicity of new compounds at an early stage in the screening procedure, a previously developed cytotoxicity assay (2) was modified and employed. Eight mammalian cell lines were used to determine the cytotoxic activity of six commercially available trypanocides and four experimental compounds. Cytotoxic activity of drugs was quantified after incubation of mammalian cells and trypanosomes for 72 h either by fluorescence or by staining with sulforhodamine B (2). The in vitro selectivity index (defined as the ratio of EC<sub>50</sub> of mammalian cells to EC<sub>50</sub> of trypanosomes) of currently used drugs was in the range of 1,500 (for the arsenical drug melarsoprol) up to 10,000 (for suramin). For experimental compounds the index was 0.0016 for colchicine up to 33,000,000 for sinefungin. The results obtained demonstrated that the in vitro selectivity index does not allow extrapolation to dosages to be administered in vivo. However, the assay can assist to identify compounds which are toxic to parasitic but not to host cells, and which should be evaluated further in animals.

- (1) Kaminsky and Brun. Acta Tropica 54 (1993) 279-289.  
(2) Skehan et al., J. Nat. Canc. Inst. 82 (1990) 1107-1112.

**T-1034** Influence Of Electromagnetic Fields (EMF) Upon The Transformation Of *Trypanosoma cruzi* To The Infective Stage For Vertebrates. S.M. Krassner<sup>1</sup>, B. Granger<sup>1</sup>, and E. ELMORE<sup>2,3</sup>. <sup>1</sup>Department of Developmental and Cell Biology, <sup>2</sup>Division of Hematology Oncology, University of California, Irvine CA 92717. <sup>3</sup>National Institute for the Advancement of In Vitro Sciences, Irvine, CA 92715.

A project was initiated to identify quantifiable, reproducible *in vitro* cellular markers for the assessment of EMF effects on living tissue. Our initial study indicates that EMF can affect the *in vitro* transformation (metacyclogenesis) of *Trypanosoma cruzi*, the etiological agent for Chagas' Disease. This crucial cytodifferentiating step in the parasite's life cycle is sensitive to a number of environmental influences, including ambient temperature and CO<sub>2</sub> level. A device programmed to emit a predetermined pattern of EMF, was tested for its effect on *T. cruzi* metacyclogenesis. Cells induced to undergo *in vitro* transformation by 5.0% CO<sub>2</sub> were exposed to the device and the percent metacyclogenesis, as well as cell division, was compared with control organisms maintained in the absence of the EMF units. Depending upon the location of the cells in relation to the device, we found that the emitted EMF pattern either potentiated or inhibited their rate of transformation to the infective state. Cells placed directly next to units showed significantly higher transformation percent rates than did control organisms (67.29 ± 1.0 S.E. vs. 63.25 ± 1.2, P < 0.05). Cells placed above and/or below the units exhibited significantly reduced trans-

formation rates ( $44.6 \pm 0.6$  and  $32.7 \pm 1.4$  respectively) when compared with control organisms ( $P < 0.001$ ), as well as with parasites placed directly next to the EMF device ( $P < 0.001$ ). Exposure to the EMF pattern did not affect *T. cruzi* cell growth. No significant changes in ambient temperatures was induced by the emitted EMF fields, suggesting that the transformation rate changes did not result from increased temperature.

**T-1035** Alternative Test Methods For The Detection Of *Clostridium difficile* Toxin For The Budget-Minded Laboratory. K.C. KALMUS, J.F. Kenny, and \*G.W. Kalmus. Dept. Pediatrics and \*Biology, East Carolina Univ. School of Medicine, Greenville, NC 27858.

The "gold standard" for diagnosis of *C. difficile*-associated disease or pseudomembranous colitis has classically been the cell culture cytotoxicity assay using human embryonic fibroblast cell lines (MRC-5 and HEF). Recently laboratories have used more rapid detection assays while employing the cell culture method for confirmation. This study evaluates two cost effective methods: a cytotoxicity assay using Hep-2 epithelial cells and a micro-volume rapid latex assay. Stool specimens were diluted 1:3 in phosphate buffered saline (PBS), centrifuged, and 1 ml aliquots from 70 random specimens were diluted 1:1 in both PBS and latex kit buffer for testing. Specific antitoxin-neutralized and unneutralized diluted samples were inoculated into 35-70% confluent Hep-2 cells and day 9-18 HEF cells. Cultures were incubated and read for cytotoxic effect at 24 to 96 hours. Micro-volume latex reagents and samples were dispensed onto multi-well glass slides using a micropipettor and results were compared to the standard latex kit procedure. Cytotoxicity assay results using <75% confluent Hep-2 cells showed no disparity with HEF cells when read beyond 24 hours. Agreement between the micro-volume and standard latex assay was 98.5% with a sensitivity of 86% and 79%, respectively, and a specificity of 98% for both methods compared to the cytotoxicity assay. The modified methods examined in this study do not compromise accuracy, sensitivity or specificity of results. The Hep-2 cells are readily available, easily propagated and cost less. The proposed micro-volume latex agglutination assay produces less bio-waste and requires the purchase of fewer kits. We recommend using <75% confluent Hep-2 cells for the detection of *C. difficile* toxin and confirming all positive and non-specific latex reactions for cytotoxicity.

**T-1036** *In Vitro* Cytotoxicity Of Dichlone. H. BABICH<sup>1</sup>, L. Blau<sup>1</sup>, E. Borenfreund<sup>2</sup>, and A. Stern<sup>3</sup>. <sup>1</sup>Stern College, New York, NY 10016; <sup>2</sup>The Rockefeller University, New York, NY 10021; and <sup>3</sup>NYU Medical Center, New York, NY 10016.

The cytotoxicity of the fungicide, dichlone (2,3-dichloro-1,4-naphthoquinone), was evaluated with human endothelial ECV304 cells, human hepatoma HepG2 cells, and bluegill sunfish BF-2 fibroblasts. Cytotoxicity was unaffected by pretreatment of the cells with

dichoumarol, an inhibitor of DT-diaphorase, suggesting that the two-electron reduction of dichlone was not significant. However, upon pretreatment with buthionine sulfoximine, a glutathione (GSH)-depleting agent, the cells were hypersensitive to dichlone. This suggested that the one-electron reduction was the predominant pathway. With all cells, dichlone exposure lessened the intracellular level of GSH. Further studies with the ECV304 cells showed that exposure to other GSH-depleting agents, such as chlorodinitrobenzene and bis(chloroethyl) nitrosourea, also potentiated toxicity. Dichlone toxicity to ECV304 cells was lessened by pretreatment with (-)-2-oxo-4-thiazolidine carboxylic acid, an intracellular cysteine delivery agent. Dichlone caused leakage of lactic acid dehydrogenase from treated ECV304 cells. These data are consistent with dichlone acting as an inducer of oxidative stress. (Supported, in part, by Schering-Plough Research Institute.)

- V-1001** Detection Of Metastatic Melanoma By Reverse-Transcription PCR. R. HELLER, X. Wang, N. Van Voorhis, C.W. Cruse, and D. Reintgen. University South Florida, Department of Surgery, 12901 Bruce B. Downs Boulevard, MDC Box 16, Tampa, FL 33612.

Detection of melanoma metastasis in lymph nodes is important for the treatment and prognosis of the disease. Current methods are not sensitive enough to diagnose the early metastasis of melanoma. This study was initiated to find a rapid and sensitive method for detecting micrometastatic disease in the lymph nodes of melanoma patients. The method utilizes reverse transcription-polymerase chain reaction to detect mRNA for the enzyme tyrosinase. Lymph nodes isolated from standard dissections, are bivalved and one half subjected to routine histopathological evaluation and the other half processed for the isolation of RNA. The method detects micrometastases by amplifying tyrosinase message with the combination of reverse transcription and double round polymerase chain reaction (RT-PCR). After PCR amplification, a 207 base pair product can be detected on a 2% agarose gel. Negative controls include cell lines and primary tumors from other epithelial tumors (breast and colon). DNA contamination is ruled out by repeating the PCR amplification on positive samples without the RT step. Twenty-nine patients' lymph nodes were analyzed by both standard pathologic staining and RT-PCR. Eleven of 29 lymph nodes were pathologically positive. Nineteen were RT-PCR positive including all pathologically positive samples. Restriction enzyme cutting showed that the amplified 207bp PCR product is part of the tyrosinase gene sequence. A spiking experiment showed that one melanoma cell in 1 million normal lymphocytes could be detected. Based on these results, RT-PCR could be a powerful tool to detect micrometastatic melanoma. Although additional follow up is necessary to assess the clinical correlation of these results, the RT-PCR method appears to be a more sensitive way of detecting occult disease and may prove to be an important prognostic tool for melanoma patients.

- V-1002** Regulation Of Differentiation In A549 Malignant Pneumocytes By Paracrine Growth Factors. C. McCormick, R.I. FRESHNEY, and L. Evans. CRC Department of Medical Oncology, University of Glasgow, Scotland.

Previously, we have shown that a crude factor (FDF) derived from lung fibroblast conditioned medium (CM) induced differentiation in A549 cells, derived from a type II pneumocyte adenocarcinoma (Speirs et al., 1991, Brit. J. Cancer, 64:693). Using alkaline phosphatase activity as an alternative differentiation marker, also inducible by CM, we have now shown that interleukin-6 (IL-6), interferon- $\alpha$  (IFN- $\alpha$ ), IFN- $\alpha$ , oncostatin-M (OSM) and insulin also induce differentiation in A549. Induction is reversible, with a half-life of about 7 d, and is dependent on the presence of dexamethasone (DX). The effects of IL-6 and IFN- $\alpha$  are additive with CM, but the effect of OSM is not.

IL-6 was shown to be present in CM and the other factors, with the exception of insulin, are potential paracrine factors produced by fibroblasts, and candidates for FDF. However, disabling antibodies against IL-6, IFN- $\alpha$ , and OSM do not deplete the activity of CM. Furthermore, IL-6 and IFN- $\alpha$  increase PA activity, while FDF was shown to decrease it. We believe that FDF may be a unique factor and are attempting to confirm this by further purification.

- V-1003** DNA Fingerprinting Of Human Tumor Lines Using Minisatellite Probes. Y.A. Reid, P. McClintock, C. WHITE, and M. Rossano-Theurer. Cell Culture Department, American Type Culture Collection, Rockville, MD 20852.

DNA fingerprints of 110 unrelated and 25 related human tumor lines were derived using three single-locus probes for hypervariable regions. The number of fragments generated by digesting genomic DNAs with the restriction endonuclease *Hinf*I and hybridizing to the probes pYNH24 (D2S44), pCMM86 (D17S74) and pCMM101 (D14S13) were 49, 55, and 59, respectively. The frequency of each fragment generated ranged from 0.004 to 0.1 with an average frequency of 0.02. The average percent difference (APD), which is a measure of the genetic relatedness among cell lines, was 83% for unrelated cell lines compared to 4.5% for related cell lines. The estimated average frequency of DNA fingerprints for the population was  $5.2 \times 10^{-7}$ . This value approximates the probability that two cell lines, selected at random from a population of unrelated human tumor cell lines, will have matched fingerprints. This procedure provides a simple, reproducible, easily interpreted method for the identification and individualization of human tumor cell lines.

- V-1004** Transformation Of Lymphatic Endothelial Cells By Infection With SV40 DNA. L.V. LEAK. Ernest E. Just Laboratory of Cellular Biology, Department of Anatomy, College of Medicine, Howard University, Washington, DC 20059.

Lymphatic endothelial cells (LEC) were isolated from sheep mesenteric lymphatic vessels and cultured in M-199 containing heparin and endothelial cell growth supplement (Anat. Rec. 235, 641, 1993). Primary cultures were subcultured in 100 mm culture dishes and used at passages 3-5 for transfection. Cells were transfected with SV40 DNA (strain 776) using calcium phosphate precipitation. A population of rapidly growing cells were then subcloned and designated SLECT-1. These cells exhibited and enhanced growth potential when cultured in minimal essential medium (MEM) containing 5-10% serum without growth factors. At confluence, the cells continued to proliferate and formed multi-layered colonies. When maintained for 6-8 weeks the cells formed tube-like structures reminiscent of lymphangiogenesis in vitro. This transformed cell line also produced and secreted plasminogen activators (tPA and uPA). SLECT-1 which exhibits enhanced proliferative potential without growth factors provides a

lymphatic endothelial cell system that will be useful for investigating various lymphatic endothelial cell functions. (Supported in part by MCB-8916625 from NSF).

**V-1005** Specific Binding Of GS-I-B4 Lectin To Cultured Human Malignant Mesothelioma Cells - Isolation And Partial Characterization Of Mesothelioma Specific Glycoproteins. K.H. BERGHÄUSER, B. Knoblauch, D. Linder\*, C.M. Heinrichs, and A. Schulz. Institut of Pathology, Institut of Biochemistry\*, University of GieBen, Germany.

Because the incidence of mesothelioma is increasing, the culture of mesothelioma cells as a source for diagnostic and therapeutical studies is becoming more important. There is still a lack of methods for fast and early characterization of cultured cells regarding malignancy and mesothelial differentiation. Lectin binding of 7 mesothelioma cell lines characterized by immunocytology, electronmicroscopy, and cytogenetic investigation were compared to lectin binding of 6 cultures of normal mesothelial cells and 4 different fibroblast cultures from different donors, established from 15 different patients with lung carcinomas. 12 biotinylated lectins, specific for different carbohydrate groups, WGA, BPA, GS-I, PNA, DBA, SBA, WFA, ABA, UEA-I, LPA, LFA, and BPA (from EY-laboratories) were tested. Out of this panel of lectins only GS-I showed a positive reaction with all mesothelioma cell lines and no binding with normal mesothelial cells, fibroblasts, or carcinoma cell lines. By affinity chromatography of solubilized mesothelioma cells on GS-I-Sepharose and elution with melibiose, 3 proteins were obtained, with apparent molecular weights of approximately 205, 110, and 54 kD as shown by a SDS-PAGE under reducing conditions. Western blot of the eluate and of solubilized cells both showed reactions of GS-I in the range of the separated glycoproteins, suggesting a high purification of GS-I-binding molecules by one step. First attempts at NH<sub>2</sub>-terminal sequence analysis failed possibly because of blocked termini. Studies with deglycosylation and peptide-mapping being done. Reports of GS-I-binding in human cells and tissue are rare in the current literature, colonic carcinomas and Ehrlich's ascites cells are the only reported human cells binding to GS-I, hence indicating the existence of tumor specific carbohydrate structure.

**V-1006** Differential Expression Of Genes Regulating Redox State During In Vitro And In Vivo Aging. BART KEOGH<sup>2</sup>, R.G. Allen<sup>1</sup>, Glenn S. Gerhard<sup>1</sup>, Robert Pignolo<sup>1</sup>, Joseph Horton<sup>1</sup>, and Vincent J. Cristofalo<sup>1</sup>. <sup>1</sup>Center for Gerontological Research, The Medical College of Pennsylvania, Philadelphia PA 19129; <sup>2</sup>University of Pennsylvania, School of Medicine, Philadelphia, PA 19103.

The cellular generation of oxygen-centered free radicals has been widely thought to play a role in the aging process. There are a number of genes whose products are important in the metabolism of reactive oxygen species. Among these are the superoxide

dismutases (SODs), catalase, glutathione peroxidase and gamma-glutamylcysteine synthetase. SODs remove superoxide free radicals from cells, generating H<sub>2</sub>O<sub>2</sub> as a product. We have examined SOD-2 activity, mRNA abundance and transcription rate in proliferatively young and senescent WI-38 cells and in 29 skin fibroblast lines established from fetal (12-20 gestational wks), young (17-34 yrs) and old (78-94 yrs) donors. Our results reveal an increase in SOD-2 mRNA abundance and transcription with proliferative age in WI-38 cells and with donor age in skin lines established from postnatal donors. While the mRNA abundance of SOD-2 determined in the skin fibroblast lines generally correlates with variations observed in enzyme activity in the different lines, the increase in SOD-2 mRNA observed during proliferative aging is not associated with any change in enzyme activity. Catalase is a heme-containing enzyme that catalyzes the dismutation of hydrogen peroxide to water and O<sub>2</sub>. We have also found an increase in steady state levels of catalase mRNA and transcription levels in postnatal lines when compared to fetal lines. We are currently analyzing the expression and enzyme activity of other genes which play a role in regulating redox state. Human skin fibroblasts derived from donors of different ages may provide a useful model for the study of development and aging changes in free radical metabolism in humans. (Supported by USPHS Grant AG00378.)

**V-1007** Lifespan Of Bovine Mammary Epithelial Cells In Vitro. G.C. BUEHRING<sup>1</sup>, R.L. Neiswander<sup>2</sup>, G.L. Niemann<sup>1</sup>, C.A. Sweeny<sup>3</sup>, M.F. McGrath<sup>3</sup>, and F.L. Schanbacher<sup>2</sup>. <sup>1</sup>School of Public Health, University of California, Berkeley, CA 94720; <sup>2</sup>Department of Dairy Science, Ohio State University, Wooster, OH 44691; <sup>3</sup>Monsato Agricultural Co., Chesterfield, MO 63198.

The fibroblast has been the traditional cell type used to study senescence. More recently epithelial cells have been utilized. Little, however, has been done to determine the lifespan of cultured secretory mammary epithelial cells and their potential as in vitro models for senescence. We initiated 12 bovine mammary epithelial cell (BMEC) cultures from either milk, or enzyme-digested explants. Cells were maintained until senescence in Dulbecco's Modified Eagle's Medium (high glucose) supplemented with 5% fetal bovine serum, insulin, and antimicrobials. Mean cell volume was determined as packed volume of a cell suspension/cell number. The secretion of alpha-s<sub>1</sub>-casein, a marker for lactating mammary gland, and lactoferrin, a marker for involutinal or sublactating gland, was determined by ELISA of spent media from lactogenic hormone-treated cultures grown on collagen gels. The highest population doubling level (PDL) reached before senescence averaged 36.3 for explants and 17.1 for cells from milk. Mean cell volume increased with increasing PDL. Alpha-s<sub>1</sub>-casein levels were considerably lower than those reported for primary cultures, whereas lactoferrin levels were about the same or slightly lower. Although BMEC at PDLs subsequent to primary culture, retain the capacity to secrete milk products, they may not attain in vivo levels because of the lack of a critical chemical factor or associated cell

type present in vivo. Our results suggest that BMEC might be an interesting in vitro model for investigating the senescence of glandular epithelium.

**V-1008** Transfection Of Fetal Human Articular Chondrocytes By SV40 Large T Antigen: Increased Life Span And Modification Of Collagen Phenotype. B. BENOIT, S. Thenet-Gauci, P. Penforis, S. Demignot, and M. Adolphe. Laboratory of Cellular Pharmacology, Ecole Pratique des Hautes Etudes, 15 rue de l'Ecole de Médecine, Paris 75006, France.

The development of an immortalized human articular chondrocyte cell line would be a valuable tool for toxicopharmacological studies. For this purpose we transfected femoral head chondrocytes obtained from a 13-week fetus with SV40 large T or temperature sensitive SV40 large T. Two months later, proliferating clones emerged and have been expanded.

To date, the transfected cells are proliferating with a constant growth rate without crisis. Control cells have been propagated in culture and rapidly showed a senescent phenotype.

Immunofluorescence staining of the clones with antibodies specific for SV40 large T antigen demonstrated the production of SV40 large T protein.

Type II and type I collagen immunolabeling appeared altered compared to primary culture. Northern blotting confirmed the disappearance of type II collagen. Collagen phenotype expression was also studied by SDS-PAGE of the intact chains of collagen after tritiated proline incorporation. The pattern showed type I collagen synthesis. 2D-electrophoresis of the peptides obtained after CNBr cleavage is in progress, and will permit to evaluate more precisely in the types of collagen synthesized.

As we recently showed that 3-D culture in alginate beads was able to re-differentiate human articular chondrocytes dedifferentiated by subculture, we intend to evaluate the collagen phenotype of the transfected fetal chondrocytes under these optimal culture conditions.

**V-1009** Establishment And Characterization Of Immortalized Clones Of Acinar Cells From Adult Rat Parotid Glands. K.N. Prasad, S. Kumar, E. Carvalho, J. Edwards-Prasad, and F.H. La Rosa. Center for Vitamins and Cancer Research, Department of Radiology, University of Colorado Health Sciences Center, Denver, CO 80262.

The purpose of this study was to establish immortalized clones of acinar cells from adult rat parotid glands. Rats were injected with isoproterenol (20 mg/kg of body weight) to induce cell proliferation in parotid glands. After 24 hours of injection, the single-cell suspensions were prepared. The transfection of single-cell suspensions with plasmid vectors, pSV<sub>3</sub><sup>neo</sup> or pSV<sub>5</sub><sup>neo</sup> by electroporation produced a cell line (2RS), which grew with a doubling time of about 24 hours, produced T-antigen, neurofilament-160,  $\alpha$ -amylase, and  $\alpha$ -amylase mRNAs of 1106 and 702 bp. These cells when injected in either

syngeneic rats or in athymic mice did not produce tumors. When 2RS cells were cultured in a selection medium (modified MCDB-153 containing a lethal concentration of geneticin, 400  $\mu$ g/ml) for about two weeks many cells died. The surviving cells were cloned and stored. One of them (2RSG) was characterized in detail. The cells of this clone exhibited morphological and biochemical characteristics similar to those found in 2RS cell line. The clonal acinar cells may be very useful in the study of growth, differentiation, and transformation. (Supported by NIH-DE09589.)

**V-1010** Cell Growth And Hybridoma Protein Secretion In Gamma-Irradiated Animal Serum Is Equivalent To Matched Nonirradiated Control Serum. R. FESTEN, B. Alderete, J. Doak, J.D. Keathley, and D.E. Wyatt. JRHBiosciences, Inc., 13804 W. 107th Street, Lenexa, KS 66215.

The risk of microbial contamination from animal-derived serum products is an increasing concern of biopharmaceutical manufacturers producing vaccines and biotherapeutics. Several methods have been explored as a means of inactivating potentially hazardous contaminants. We have previously shown that under controlled conditions, it is possible to effectively inactivate virus, fungi, phage and bacterial contaminants in fetal bovine serum (FBS) while maintaining the growth promoting properties of the sera<sup>1,2</sup>. For this current study, donor horse serum (DHS) and FBS were gamma-irradiated under controlled conditions and evaluated relative to matched unirradiated control serum. Multiple passage cell growth assays in triplicate 25 cm<sup>2</sup> flasks, clonal growth and plating efficiency tests were performed to evaluate the nutritive growth properties of the irradiated DHS. Monoclonal antibody production in the presence of irradiated DHS and FBS was quantitated. In media containing 10% DHS, cell growth of 3T3 fibroblasts, Vero green monkey kidney, Sp2/0-Ag14 myeloma and L243 hybridoma cells achieved densities greater than 84% of matched nonirradiated controls. Plating efficiency assays of Vero cells and cloning assays using Sp2/0-Ag14 and L243 showed satisfactory results, i.e.  $\geq$ 75% of nonirradiated control, in all tests. Monoclonal antibody production by L243 cells in three lots of FBS was equal to or greater than control samples at 10 and 2% serum supplementation. L243's in 10 and 5% DHS showed equivalent antibody production to matched unirradiated DHS. We have shown that DHS can be gamma-irradiated at doses shown to inactivate a wide variety of contaminating organisms without forfeiting the growth promoting qualities of the serum. In conjunction, we also show that the level of immunoglobulin secreted by a hybridoma cell line remains uncompromised when harvested from growth media containing irradiated serum.

<sup>1</sup>BioPharm, June, pp 34-40, 1993.

<sup>2</sup>BioPharm, July/Aug, pp 46-52, 1993.

**V-1011** Magnesium, Glutamine, And Serum As Interactive Agents In The Anchorage Independent Colony Formation Of Rat Liver Epithelial Cells. BRUCE S. HASS, Patrice L. McDaniel, and Neil A. Littlefield. NCTR, Jefferson, AR 72079.

The lack of magnesium (Mg) retards the growth of extant tumors as well as potentiates the growth of tumors in non-tumorous tissue. In attempting to mimic these effects *in vitro*, we used non-transformed (ARL15) and transformed (TRL8) rat liver epithelial (RLE) cells in the soft agar assay in the presence of three medium variables: glutamine (gln) as an energy source; fetal bovine serum as a growth factor source; and Mg. The cells were grown in Williams medium E at 0, 1, or 2x the standard [Mg] of 97.7 mg/L; 3 or 10% v/v serum; and 1.3 or 2.0x the standard conc of gln (292 mg/L). After one passage (~1 week) in treatment medium the cells were plated in 0.33% agarose containing the same concs of treatment nutrients. The frequency of soft agar colonies was determined in duplicate experiments; the data are tabulated in terms of the ratio of colony frequency of (TRL8) to that of (ARL15) at various [gln], [serum], and [Mg]:

	(I) TRL8/ARL15 @ 1.3x[gln]		(II) TRL8/ARL15 @ 2.0x[gln]	
	3% Serum	10% Serum	3% Serum	10% Serum
0x [Mg]	1.8	1.5	19.7	7.4
1x [Mg]	3.0	4.1	6.4	0.7
2x [Mg]	3.8	24.0	22.4	11.3

Results: (1) At the lower [gln] (I): (a) The presence of Mg in normal cells inhibits transformation, and its absence enhances the transformation response; (b) The presence of Mg in transformed cells enhances transformation while its absence reduces it; (c) Higher [serum] tend to enhance the transformation response. 2) At higher [gln] (II) the transformation response is different than at 1.3x [gln] (I): (a) A bimodal response occurs with a minima at 1x [Mg]; (b) Higher [serum] suppresses the transformation response; (c) The transformation response is higher in II than I except for 1x and 2x[Mg] at 10% serum (0.7 v 4.1 and 11.3 v 24.0). Conclusion: since gln is an energy source for cells *in vitro*, transformation is a function of the energy available to the RLE cell and that interactions among transformation-dependent factors such as the growth/inhibition components of serum and Mg with such energy components contribute directly to the complex transformation response.

**V-1012** Regulation Of Ciliated Cell Differentiation In Cultures Of Rat Tracheal Epithelial Cells. A.B. CLARK, T.E. Gray, T. Bader, P. Nettesheim, and L.E. Ostrowski. Laboratory of Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC 27709.

Ciliated cells, by virtue of their essential role in mucociliary clearance, are an important component of the lung defense mechanisms. Ciliated cells are sensitive to a range of air pollutants and are damaged or lost in a variety of airway diseases. The repair of damaged

airway epithelium requires replacement of the ciliated cells through differentiation of precursor cells. We are studying the regulation of ciliated cell differentiation using the *in vitro* model system developed in our laboratory by Kaartinen et al. (*In Vitro Cell. Dev. Biol.*, 29A:481, 1993) in which primary rat tracheal epithelial (RTE) cells plated on collagen coated membranes develop into a mucociliary epithelium which resembles the *in vivo* trachea. To begin to investigate factors that may regulate ciliated cell differentiation, we examined the effects of several media components for their effect on the extent of ciliogenesis. Maintenance of the cultures in the submerged state, as has been previously reported, significantly reduced the number of ciliated cells. A decrease in ciliated cell number was also observed if hydrocortisone was omitted from the media. Withdrawal of growth promoting factors from the media, including bovine pituitary extract, epidermal growth factor, or cholera toxin, resulted in a 2- to 3-fold increase in the percentage of the culture surface covered by ciliated cells. A further increase in the percentage of ciliated cells was observed when combinations of these factors were removed. Cell number was reduced about 50% by removal of growth promoting factors during the culture period studied. These results suggest that high levels of growth promoting agents, required during the early culture period, may inhibit differentiation into ciliated cells.

**V-1013** Modulation Of Angiogenesis By Anti-Fibroblast Growth Factor Monoclonal Antibody. M.E. Schelling, S. Venkateswaran, V. Blanckaert, and A. Zijlstra. Department of Genetics and Cell Biology, Washington State University, Pullman, WA 99164-4234.

Anti-Fibroblast Growth Factor (FGF) receptor monoclonal antibodies (Mabs) were produced by *in vitro* immunization against the native *flg* FGF receptor. Mab VBS1 treatment of endothelial cells (ECs) resulted in blocking <sup>125</sup>I-FGF binding, modulation of the proliferation of ECs and their differentiation into tubules ("in vitro angiogenesis"), and phosphorylation of the FGF receptor at 120 kDa. Both the bivalent (F(ab')<sub>2</sub>) and monovalent Fab' Mab fragments inhibited <sup>125</sup>I-FGF binding. The monovalent Fab', however, did not result in receptor autophosphorylation. Addition of anti-F(ab')<sub>2</sub> antisera, which crosslinked the Fab'-receptor complexes, restored receptor autophosphorylation. These data suggest that receptor occupancy by the ligand is not sufficient for signal transduction, and that FGF receptor dimerization or clustering is required.

**V-1014** Transplantation Of A Bioengineered Bone Marrow Tissue And Its Potential Use As A Substrate For Toxicity Assessment. B.A. NAUGHTON, J. San Román, B. Sibanda, J. Gee, D. Morales, and V. Kamali. Advanced Tissue Sciences, Inc., La Jolla, CA 92037.

We developed a transplantable bone marrow (BM) tissue by co-culturing BM stromal cells (fibroblasts, adipocytes, histiocytes, endothelia, etc.) with various hematopoietic cell populations of Long-Evans rats on

three-dimensional templates composed of filaments of polyglycolic acid (PGA), a bioresorbable material. BM stromal cells were cultured for 7 days after seeding onto PGA felt constructs and then were inoculated with fresh, non-selected BM cells or various hematopoietic cell populations that were selected using monoclonal antibodies and cultured for an additional 15 days. Extensive, disseminated hematopoiesis was observed at the graft sites by as early as 30 days post surgery. BM tissue became integrally organized at the graft sites and persisted for at least 6 months *in situ*. Multilineage hematopoiesis and survival of the Thy 1.1 cell population (stem/progenitor cells) at the graft sites was confirmed by immunohistological analysis. In contrast to conventional BM transplants, this method permits the transplant of BM hematopoietic cells as a tissue (i.e., with intact communication with the full complement of BM stromal support cells). As such it may be applicable for the treatment of conditions associated with microenvironmental BM defects or in those experiencing graft failure. In addition to potential therapeutic applications, we also tested the influence of several drugs on BM that was co-cultured on nylon screen templates. Most of the agents induced a dose-related diminution in the concentration of myeloid/macrophage hematopoietic progenitor cells present in the BM co-cultures. In addition, certain drugs exhibited differential lineage specificity as evidenced by phenotypic analysis indicating the potential utility of this model for drug screening.

**V-1015** Effects of Trypsin On The Surface Architecture Of Embryonic Spinal Ganglion Cells In Vitro. E. Lindner, N. Inczedy-Marcsek, L. HSU\*, F. Miragall, and R. Dermietzel. Department of Anatomy, University of Regensburg, Germany; \*Department of Biology, Seton Hall University, South Orange, NJ 07079.

To record early morphological and immunocytochemical changes in cell surface architecture, whole chick embryonic spinal ganglia of different ages (4-8 d) or dissociated monolayer cultures were exposed to 0.25% trypsin for 10, 20, 30, and 40 min intervals and examined by scanning and transmission electron microscopy. Cryostat sections of ganglia were treated with 0.01% trypsin and incubated with primary antibodies including both the adult and the polysialylated embryonic forms of neural cell adhesion molecule (NCAM), the L1 glycoprotein, laminin, and fibronectin. Trypsin treatment elicited the formation of fingerlike microvilli processes and micro- and macro-pinocytotic pits from the neuronal and glial soma. In contrast, the surfaces of untreated neurons and neurites remained smooth. Immunodetection for both forms of NCAM, L1, or laminin was not different in trypsin-treated ganglia tissue but a loss of fibronectin was observed. Our results indicate that the enzymatic dissociation of primary neuronal tissue is accompanied by rapid morphological surface alterations and loss of specific extracellular matrix component. Such changes may impact on the subsequent response of cultured neuronal cells to biologically active agents such as nerve or epidermal growth factors which are commonly used to analyze differentiation.

**V-1016** Culture Of Neuronal Cells From Zebrafish Embryos. C. GHOSH and P. Collodi. Department of Animal Sciences, Purdue University, West Lafayette, IN 47907.

The zebrafish (*Brachydanio rerio*) is a popular nonmammalian model of vertebrate development providing some advantages over mammalian models. Virtually all studies with zebrafish have employed *in vivo* approaches and practically no work has been directed toward *in vitro* approaches utilizing zebrafish cell culture systems. This report describes the culture of neuronal cells derived from zebrafish blastula-stage embryos. The zebrafish blastula is comprised of pluripotent cells. Under the culture conditions that we have developed, the blastula-derived cells undergo neuronal differentiation. The embryo cells were cultured in nutrient medium supplemented with insulin (10 µg/ml), trout serum (0.4%), trout embryo extract (40 µg/ml), and fetal bovine serum (1%). Poly-D-lysine substrate stimulated the appearance of neuronal cells in the embryo cell cultures. The neuronal cells were identified by their morphological, immunological, and neurochemical characteristics. Neuronal differentiation was apparent in 72 h cultures from the presence of neurite outgrowth. Extensive neurite branching was observed after 2 weeks in culture. The embryo cells also possessed high levels of the neuron-specific enzyme, acetylcholinesterase. Enzyme levels increased approximately 40-fold after 2 weeks in culture. Western blot analysis revealed that the cells expressed a 200-kd protein recognized by antineurofilament polyclonal antibody. The zebrafish embryo cell cultures provide an *in vitro* system to investigate the genetic and biochemical parameters influencing neuronal cell growth and differentiation.

**V-1017** The *De Novo* Activation Of The Vitellogenin Gene Family Is Accompanied By A Spatial Rearrangement Of Centromeric Domains. J. JANEVSKI, P.C. Park, and U. De Boni. Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Chromatin domains within interphase nuclei of several cell types exhibit a distinct, non-random spatial organization. The existence of a function-dependent organization is supported by evidence which suggests that specific chromatin domains undergo spatial rearrangement under conditions which alter gene expression. Exposure to estrogen of male *Xenopus laevis* hepatocytes *in vitro* results in *de novo* activation of vitellogenin mRNA production and vitellogenin protein synthesis. In a test of the hypothesis that the *de novo* induction of vitellogenesis in male *Xenopus laevis* will be associated with a spatial rearrangement of specific chromatin domains, centromeric regions were localized by immunofluorescent labeling of associated kinetochore proteins. Induction of vitellogenesis was confirmed by immunofluorescence for vitellogenin protein. The 3-D spatial distribution of kinetochores was determined by optical sectioning of interphase nuclei using confocal laser scanning microscopy, and changes in the distribution of kinetochores were assessed as a function

of time of exposure to estrogen. Analyses of kinetochore positions revealed that their spatial distribution in naive male hepatocytes was significantly different than that observed in estrogen treated vitellogenic hepatocytes. Kinetochores in vitellogenic male cells exhibited a significantly greater association with the nuclear periphery than did non-estrogen treated cells. Such rearrangements were evident within 24 hours of estrogen exposure. In summary, the results show that centromeric domains within interphase nuclei of *Xenopus* hepatocytes undergo spatial rearrangement under conditions which alter the transcriptional state of the cell.

**V-1018** Coculture Of Epithelial And Stromal Endometrial Cells Provides Model For Study Of Cell-Cell Interactions In Neoplasia. J.T. ARNOLD, J.F.H. Blasser, and D.G. Kaufman. Department of Pathology, University of North Carolina, Chapel Hill, NC 27599-7525.

A coculture system has been developed using human endometrial stromal and epithelial cells separated by filter inserts. Media has been optimized for the coculture of both cell types and extracellular matrix substrata has been optimized for basement membrane production and differentiated cell morphology in the epithelial cells. Fetal epithelial endometrial cells, previously immortalized by an origin-defective SV40 construct, were cocultured with normal stromal cells and compared to cocultures of adenocarcinoma cells with normal stromal cells. Studies were conducted to determine steroid receptor status using cells unstimulated by steroids. Analysis of messenger RNA by reverse transcription and PCR indicate differential expression of steroid receptors in normal versus transformed cell cocultures as well as in subconfluent versus confluent cultures. We have also noted different estrogen receptor isoforms, expressed by different size PCR products, between the epithelial and stromal cell types. This model will be useful in identifying factors involved in stromal-epithelial communications, especially those involved in the progression of neoplasia.

**V-1019** Effect Of Dichloroacetic Acid, Trichloroacetic Acid And Chloral Hydrate On Intercellular Communication In Clone 9 Rat Hepatocyte Cells. S.G. BENANE, C.F. Blackman, and D.E. House. Health Effects Research Laboratory, Research Triangle Park, NC 27711.

The introduction of chlorine in water purification processes has created a whole new problem concerning the safety of our drinking water. When chlorine reacts with organic matter in raw water, chloroacetic acids and their corresponding chloroacetaldehydes are formed. Finished drinking water has been found to contain both of these in the  $\mu\text{g/L}$  range. Dichloroacetic (DCA) and trichloroacetic (TCA) acids, have been found to be carcinogenic in mice. Trichloroethylene (TCE), a commonly used industrial solvent, is metabolized in rodent liver to TCA and trichloroethanol (TCEth) possibly through a chloral intermediate (chloral hydrate [CH]). Gap junction intercellular communication (IC) appears to be necessary for cells to function normally, which includes

the control of cell division. Further, inhibition of IC is thought to be important in nongenotoxic carcinogenesis. We used Lucifer Yellow scrape-load dye transfer as a measure of IC to look at the effect of DCA, TCA, and CH on Clone 9 cell cultures (normal rat hepatocytes). Initial screens were performed to determine the concentrations of each chemical that would cause changes in pH and cell toxicity, as a function of duration of exposure, and the concentration range that reduced IC. From these data, concentrations were chosen for further detailed study. Four independent experiments were done for each chemical using exposure times of 1, 4, 6, 24, 48, and 168 hours. For DCA, doses of 0, 5, 10, and 50 mM were used. There was no difference in IC between controls and 5 mM for any of the time periods. There was a difference in IC between controls and 50 mM for all time periods. IC in cells exposed for 6 h at 10 mM, were different from controls. For TCA, doses of 0, 0.5, 1, 2.5, and 5 mM were used. There was no difference in IC between controls and 0.5 mM for any of the times. At 1 and at 168 h there was also no difference in IC between 1 mM and controls. For all other doses and times the IC was different from controls. For CH, doses of 0, 1, 5, and 10 mM were used. For 1, 4, and 6 h there was no difference in IC between 1 mM and controls. For all other doses and times there was a significant difference in IC compared to controls. Analysis of variance and REGW multiple comparison procedures were used to analyze all data. The lowest concentration and shortest time that DCA significantly reduced dye transfer was 10 mM at 6 h. For TCA, it was 1 mM at 1 h, and for CH, it was 1 mM at 24 h. When converted to  $\mu\text{g/L}$ , all doses that produced significant changes in IC are well above that found in drinking water.

This abstract does not necessarily reflect EPA policy.

**V-1020** Growth Of Microvascular Retinal Cells In Serum-Free Media. I.M. EVANS, L.F. DeTulleo, H.L. Huyck, S.J. Leuenroth, J.E. Maul, C.M. Stocum, and J.F. Walter. Biology Department, Rochester Institute of Technology, Rochester, NY 14623.

Bovine retinal capillary endothelial (BRCE) and pericyte (BRP) cells were grown in serum-free medium to facilitate the study of secreted growth factors. Microvascular cells, isolated from bovine retinae, initially were plated on fibronectin-coated cover slips and maintained in 20% fetal bovine serum-containing medium. BRCE colonies were identified initially based on their regular polygonal shape. Putative BRCE colonies were identified by staining for factor VIII related-antigen-von Willebrand factor and by uptake of acylated LDL. Irregular BRP colonies containing finger-like projections were identified by actin staining. Both BRCE and BRP cells were maintained in several commercially available serum-free media as well as Ham's F12 supplemented with insulin, transferrin, and selenium (HITS). BRP cells were passaged in these media; a doubling time of 1-2 days was measured. BRCE cells underwent at least one passage in HITS supplemented with fibroblast growth factor. Growth, however, was very slow; a doubling time of 5-7 days was found. Improvements in a serum-free

medium for BRCE and BRP cells should facilitate the study of growth factors secreted by these cells.

- V-1021** Ion Transport Across A High-Resistance Blood-Brain Barrier Examined By Non-Invasive Voltage Probes. P.J.S. SMITH and A.M. Shipley. NVPF, M.B.L., Woods Hole, MA 02543.

Where brain microenvironments differ significantly from the blood ion levels, animals have high-resistance blood-brain barriers, a condition exemplified by vertebrates and insects. Insects, with ganglionic resistances of 1200Ωcm, provide experimental models of tight barriers and have been used extensively to study development and modulation. However, as the cells comprising the barrier are thin and attenuated, electrophysiological studies have been difficult. In 1990, the authors explored the use of a non-invasive voltage sensitive probe (NVP<sub>PD</sub>) to map the net ion flux across the barrier. This technique demonstrated an outwardly directed current of approximately 3-4 μA.cm<sup>-2</sup> across the ganglionic barrier. Subsequent work combining a pharmacological approach with the NVP<sub>PD</sub> and using the newly developed non-invasive ion-selective voltage probe (potassium: NVP<sub>K</sub>) indicated that the major ion involved was potassium but that the motive force behind this flux was an unusual plasma ATPase. The net potassium flux across the barrier was sensitive to mitochondrial ATPase inhibitors such as oligomycin but was not shut down by the application of either ouabain or vanadate. N-ethylmaleimide did inhibit the current indicating the involvement of a nonphosphorylated vacuolar-type H<sup>+</sup>-ATPase (V-type ATPase), perhaps transporting potassium via a H<sup>+</sup>/K<sup>+</sup> antiport. The high concentration of mitochondria in the ganglionic sheath cells and their extensively folded membrane imply that the pumps are located in the outermost layer of the barrier.

- V-1022** β<sub>2</sub>-Microglobulin-Dependent Positive Co-Operation Between Antigen-Presenting Cells. U. ARMATO, J. Wu, and D. Barisoni. Institute of Anatomy and Histology, University of Verona, I-37134 Verona, Italy.

Antigen-presenting cells (APC) play important roles in the allo-reactive proliferation of T cells in vitro and in the allo-graft rejection reactions in vivo. However, the possibility of a cooperation between donor's APC (dAPC) and recipient's APC (rAPC) was not previously investigated. Hence, the present work was aimed at establishing whether a synergism of any kind occurs between dAPC and rAPC during allo-reactions, and whether certain functionally relevant cell surface molecules, such as β<sub>2</sub>-microglobulin (β<sub>2</sub>m) and human leukocyte antigens (HLA), are involved in such an interaction. Our results show that the in vitro allo-reactive proliferation of peripheral blood lymphocytes (PBL) is significantly intensified by a positive co-operation between dAPC and rAPC. Moreover, this same synergistic co-stimulatory activity can be markedly hindered by a monoclonal antibody aimed against the β<sub>2</sub>m, while being unaffected by the monoclonal antibodies binding to either HLA-A-B-C or HLA-DR molecules. Therefore, our findings reveal for

the first time that a hitherto overlooked, β<sub>2</sub>-microglobulin-dependent, positive interaction between dAPC and rAPC does indeed take place. This interaction is likely to be important for both the understanding and a better control of the mechanisms underlying allo-graft rejection reactions [1].

[1] J. Wu, D. Barisoni, L. Menapace, M. Ribecco, and U. Armato., *Burns*, 19:289, 1993.

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- V-1023** Non-Invasive Voltage Probe (NVP) For The Measurement Of Steady Ionic Currents. Joseph G. Kunkel, Alan M. Shipley, Richard H. Sanger, and PETER J.S. SMITH. National Vibrating Probe Facility, Marine Biological Laboratory, Woods Hole, MA 02543.

Non-invasive voltage probes for measuring net ionic currents have been available for over a decade. A similar probe that is ion selective is needed to measure the flow of specific ions more directly as well as to allow non-electrogenic phenomena such as pumps and pathways to be examined. Electrodes selective for particular ions can be fashioned to have spatial resolutions in the micron range. These microelectrodes can be oscillated short distances (e.g. 10 microns) and the recorded voltage difference from the extremes used to estimate the ion gradient. A potential from the static probe gives the gross ion concentration based on a Nernst equation calibration of the microelectrode. This when combined with the difference potential from the oscillating probe allows the estimate of ion flux at a point relative to a source or sink. Gradients of K<sup>+</sup>, Na<sup>+</sup>, H<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and NH<sub>3</sub><sup>+</sup> can each be measured in relation to cell or tissue surfaces and followed as they change in time. With this NVP<sub>1</sub>, the Facility has investigated several preparations which show patterns of ionic currents related to function. The cockroach ovarian follicle, previously studied with a vibrating voltage probe (NVP<sub>PD</sub>), has been shown with an NVP<sub>K</sub> to have a substantial potassium current emanating from the follicle cell layer at its anterior pole. A 2-dimensional version of the NVP<sub>K</sub> was used to map the pattern of K<sup>+</sup> current in this preparation. The resultant 2-dimensional data allows the extent of the active epithelium to be demarcated. Resolution is not, however, limited to relatively large systems. Localized Ca<sup>2+</sup> and H<sup>+</sup> gradients have been measured from much smaller cells - for example, single oxyntic cells, pancreatic β-cells, and isolated neurons. (Supported by NIH grant P41RR01395.)

- V-1024** Entrapment of Hepatocyte Spheroids In A Hollow Fiber Bioartificial Liver for Potential Treatment of Liver Failure. F.J. WU<sup>1</sup>, M.V. Peshwa<sup>1</sup>, F.B. Cerra<sup>2</sup>, and W-S. Hu<sup>1</sup>. Departments of <sup>1</sup>Chemical Engineering and Materials Science and <sup>2</sup>Surgery, University of Minnesota, Minneapolis, MN 55455.

A xenogeneic hepatocyte hollow fiber entrapment bioreactor has been developed as a bioartificial liver for potential treatment of acute human fulminant hepatitis. Hepatocytes cultivated as spheroids have been ob-

served to exhibit prolonged viability, enhanced liver-specific function and differentiated state compared to monolayer cultures. The feasibility of entrapping spheroids in our bioartificial liver depends upon whether the rate and efficiency of spheroid formation can be increased. Hence, it is important to understand the formation mechanistic of and cellular organization within spheroids. By staining a population of cells with the fluorescent vital dye 3,3'-diocetadecyloxycarbocyanine perchlorate (DiOC<sub>18</sub>(3)), we have tracked individual cell movement during spheroid formation by time lapse microscopy. Transmission electron micrographs indicate extensive cell-cell contact and tight junctions between hepatocytes, and bile-canalicular-like networks within spheroids. Results indicate that the cytoarchitecture of spheroids resembles that of an *in vivo* liver lobule. Collagen entrapment for three days did not appear to alter the gross organization or morphology of cells within spheroids, as seen by scanning electron microscopy. Assessment of liver-specific function within a spheroid-entrapment BAL *in vitro* indicates significant improvement in device performance compared to entrapment of a suspension of single-cell hepatocytes. A spheroid-entrapment bioartificial liver thus warrants further studies for potential human therapy.

**V-1025** A Stereotypic, Transplantable Liver Culture System. B.A. NAUGHTON, B. Sibanda, J. San Román, J. Gee, D. Morales, and V. Kamali. Advanced Tissue Sciences, Inc., La Jolla, CA 92037.

Rat hepatic parenchymal cells (PC) that were co-cultured with liver-derived stromal cells (Kupffer cells, endothelia, fibroblasts, fat storing cells, etc.) exhibited functional activity (e.g., albumin and fibrinogen synthesis, cytochrome P450 activity, etc.) for up to 7 wk when cultured on three-dimensional templates (screens or felt-like material made of nylon or polyester filaments). During the initial culture stage, stromal cells "seed" these templates and grow in a stereotypic manner. When established, they supported a second inoculum of PC. These PC maintained normal phenotypic expression and proliferated until all available space for expansion within the template was utilized. Similar observations were made using human liver cells. These functional hepatic PC:stromal cell co-cultures can be employed as fundamental components of an external "liver assist" device or to perform toxicological studies. In addition, we established liver cell co-cultures using a similar methodology on bioresorbable polymer templates composed of polyglycolic acid (PGA) and polylactic acid (PLA) fibers. After ~2 wk *in vitro* the co-cultures were transplanted into normal rats or animals that were subjected to ~40% hepatectomy (Hx). Hx rats, but not normal animals, regenerated liver architecture at hepatic and extrahepatic graft sites within 30 days; donor-derived PC produced albumin, fibrinogen and other proteins. Hepatopoietic foci diminished in area with time so that by 102 days *in situ* only isolated clusters of functional PC were still evident indicating that, at least for the survival of grafts at extrahepatic sites, continual hepatotrophic stimulation is required. This grafting method may prove

useful in treating hepatic metabolic deficiencies related to single gene defects by replacing a portion of malfunctioning liver with normal liver tissue that regenerates *in situ*.

**V-1026** New Scale-Up Technologies For Anchorage-Dependent Cell Culture. R.W. BOICE and A.J. Meuse. Costar Corporation, Cambridge, MA 02140.

Scale-up technology for anchorage-dependent cells has been largely limited to roller bottles and microcarriers, with few technology advances over the past 10-15 years. Many production scale-ups do not consider either technology viable for manufacturing. As a result, many commercial scale-ups have been forced to adapt anchorage-dependent cells to suspension culture in bioreactors to avoid the limitations of existing technologies, and to allow the addition of perfusion culture methods to improve bioprocesses. Often, this adaptation to suspension culture causes significant decreases in cell productivity, and extensive optimization.

A new modular integrated system has been developed for mass culturing of anchorage-dependent cells. The bioreactor modules are based on parallel plates of tissue culture treated styrene in a closed-loop, low-shear, perfusion system which is very compact. The system design features unique low-shear pumps, a thin-film oxygenator, and pH and O<sub>2</sub> monitoring and control capabilities.

The system is designed for the GMP production of animal and insect cells for harvest, secreted products, and viral vaccines. These modular reactors have shown superior performance in cell densities, viabilities, secreted product titres, and virus titres, with long-term stable production runs over 100 days, combined with very good cost and labor savings.

**V-1027** Production Of Human Recombinant Pro-Stromelysin I In A Baculovirus Expression System. P.R. BENTON, M. Walroth, P. Cannon, and P. Belloni. Syntex Research, Palo Alto, CA 94304.

A baculovirus (BV-rHS-1) expression vector for human recombinant pro-stromelysin (rHS-1) under the control of *polh* promoter has been generated. Clonal isolates of BV-rHS-1 were grown in SF9-SF host cells to establish working and reference stocks. A single clone (Clone 15) was expanded and used to compare expression levels generated in an SF-9 host with production from T.ni 5B1-4 (JRH Biosciences), a suspension adapted, serum-free culture host cell line. The T.ni 5B1-4 cells appeared to be more susceptible to virus infection, exhibiting 5- to 10-fold higher titers compared with SF9 titers generated in parallel studies. Further characterization of SF9-SF cultures in shake flasks indicated that optimum production of rHS-1 was achieved in cultures infected with an MOI of 1 and allowed to incubate at 27° C for 48 hours.

Immunocytochemical analysis was used to confirm expression of rHS-1 in both host cell lines. Conditioned media generated from each BV-rHS-1 infected host was analyzed for secreted recombinant stromelysin by west-

ern blotting. The amount of secreted rHS-1 was significantly higher in T.ni 5B1-4 cultures compared to SF-9 cultures at identical MOIs (300 mg/l:150 mg/l). Additional biochemical analysis suggests that rHS-1 is processed correctly in both host systems since trypsin activation was analogous to native stromelysin for each product. Due to problems of protein precipitation in T.ni 5B1-4 culture supernatants, SF9-SF cells are currently the host of choice for rHS-1 production.

**V-1028** The Hybridoma Bank: Hybridoma Cell Lines Available For Basic Research. K. STEENBERGEN and P.R. McClintock. American Type Culture Collection, Rockville, MD 20852.

The Hybridoma Bank consists of over 200 immunoglobulin-secreting cell lines available for distribution to the scientific community. The bank is operated for the National Institute of Allergy and Infectious Diseases by the American Type Culture Collection, and its purpose is to acquire, characterize, store, and distribute hybridomas that are of significant interest to biomedical researchers. The lines secrete immunoglobulins with reactivities for class I, class II and differentiation antigens of several species, viruses and microbial agents and a large number of defined proteins. New hybridomas are added to the bank through a three-stage process. Lines submitted by their developers are screened on arrival for microbial contaminants. The ability of the cells to secrete antibody of the correct isotype is verified, and the culture is expanded. After expansion and freezing, the activity and specificity is tested by the donor and, if acceptable, the line is distributed. Hybridoma Bank lines are available to all scientists for research purposes. No commercial use of the cells or their products is permitted. Technical support in the form of information about culture conditions, antibody specificity and other characteristics is provided. Tables showing the Hybridoma Bank lines along with their specificities and other characteristics are available. The Hybridoma Bank lines, together with hybridomas in our other collections, make up more than 600 lines banked at the ATCC with reactivities that span a broad range of antigens.

**V-1029** Trends In The Incidence And Distribution Of Mycoplasma Contamination Detected In Cell Lines And Their Products. V. Pawar, J. Luczak, M.S. Cox, J. Dubose, Jr., and J.W. HARBELL. Microbiological Associates, Inc., Rockville, MD 20850.

It has been established that 10 to 20% of the cell cultures in use are contaminated with mycoplasma; primary cell culture accounting for about 1%, while most occurring from exogenous source such as within the laboratory or production facility. Use of antibiotics in routine culture has led to antibiotic-resistant strains for most mycoplasma species. This has served to mask but not remove mycoplasma contamination, thus providing a reservoir for subsequent infection. All samples in this study (1985 to December 1993), were treated using two methods: agar/broth cultivation and Vero Cell Culture assay with Hoechst staining. Two levels of assays were

performed on cells and products: research level and samples for regulatory submission. Under the research level testing, 3329 cell lines were examined with 394 positive (11.8%), 296 products were tested with 6 positives (2.0%). The predominant organisms isolated were *M. arginini*, *M. hyorhinis*, and *M. fermentans*. Under the regulatory testing, 1195 cell lines were tested with 47 positives (3.9%) and 3899 samples of products (all stages) were tested with 30 positives (0.8%). The predominant organisms isolated were *M. hyorhinis*, *M. arginini*, *M. fermentans*, *M. orale*, and *M. salivarium*. Although the incidence of contaminated cell lines intended for product development has increased from 0.9% (1985-1990) to 6.5% (1991-1993), contamination of product samples have remained consistent (0.8%) over this period showing the need to also focus testing on production cell banks. The increased incidence of contamination and the species of mycoplasma involved are suggestive of the need for adequate testing and control programs on production cell banks. The data further points to cross contamination from cell cultures rather than reagents as the chief source of infection.

**V-1030** Characterization Of Clones From *Ras* (TG-AC) Transgenic Mouse Skin Tumors. J.-L. D. KLEIN, C. Szczesniack\*, R. Cannon\*, C. Trempus\*, J. Spalding\*, and J. Roberts. Laboratory of Molecular Carcinogenesis, \*Laboratory of Environmental Carcinogenesis and Mutagenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Different approaches have been used to characterize the steps in carcinogenesis. One is *in vitro* transformation, in which initiation, promotion and progression terms have been defined. Another is to select cells from tumors at different stages in the progression of the disease. We chose another approach using transgenic mice that carry the *v-Ha-ras* oncogene under the control of  $\xi$ -globin promoter. These mice, developed by Leder et al. (Proc. Natl. Acad. Sci. USA 87, 9178-9182 [1990]) and named TG-AC, are "genetically initiated" and develop skin tumors after promoting treatment such as brief application of TPA, or during wound repair. We cultivated cells from several induced skin malignancies or papillomas and obtained cells with various morphologies. Normal fibroblast, epithelial, and various types of transformed clones were observed in culture. Representative cells of each class were tested in FVB/N parental mice for tumorigenicity. In addition, the expression of the *v-ras* transgene and metastatic potential of various clones have been examined. Results of characterizations of representative clones will be presented.

**V-1031** Visualizing The Molecular Dynamics Of The Actin-Cytoskeleton In Normal And Transformed Cells Using Light-Optical-Based Reagents. K.A. GIULIANO and P.L. Kornblith. Department of Neurological Surgery, University of Pittsburgh, and Center for Light Microscope Imaging and Biotechnology, Carnegie Mellon University, Pittsburgh, PA 15213.

The chemical processes that comprise living organisms are the result of the precise orchestration of ions, metabolites, macromolecules, macromolecular assemblies, and organelles in time and space. One of these processes, the regulation of the actin-cytoskeleton, is germane to the expression of cell motility and is dictated by the assembly and crosslinking state of actin and the amount of tension exerted by the actin through the action of motor proteins. We developed light-optical-based reagents for use in live cells to begin to dissect the complex events that underlie cell motility. The reagents, many of them protein-based, are designed to take advantage of several modes of light microscopy including fluorescent analog cytochemistry, fluorescence photobleaching recovery, steady state fluorescence anisotropy, and fluorescence ratio imaging. Using these reagents and a multimode light microscope, we discovered that locomoting normal fibroblasts exhibit gradients in a) free  $Ca^{2+}$  ion concentration, b) the activation of calmodulin and its binding to target proteins, and c) the assembly of actin and its associated motor molecule, myosin II. A model for normal cell movement consistent with these results requires the precise temporal and spatial coupling of the above events. We are now applying this approach to cultured normal human glioma and glioblastoma cells to describe the role that the actin-cytoskeleton plays in the interrelated processes of tumor cell proliferation, invasion, and differentiation.

**V-1032** Development Of An Ulcerative Colitis-Derived Cell Line. G.C. BALCH, L.A. Manzano, J.S. Stauffer, and M.P. Moyer. Department of Surgery, University of Texas Health Science Center at San Antonio, TX 78284-7842.

We have established one of the first cell lines derived from the colonic mucosa of an ulcerative colitis (UC) patient. The cells were harvested from the mucosal scrapings of a surgically resected transverse colon. The line, UCC1t, was cultured in April of 1993 and began as a very slow growing suspension culture. After three months, the line began to grow as a monolayer and is now at Passage 8. UCC1t cells failed to form colonies in soft agar. Immunoperoxidase staining confirmed the epithelial origin of these cells. Vimentin (a fibroblast marker) stains were negative, while keratin (an epithelial marker) stains were 90% positive with a relative intensity of two on a scale of one to four. Immunoperoxidase staining for the expression of oncogene and tumor suppressor gene products yielded positive reactions for pan-*ras*, mutant p53 and DCC (Deleted in Colon Cancer). DCC stains were 30-50% positive with a relative intensity of two. Mutant p53 stains were 80% positive with a relative intensity of two, while pan-*ras* stains were

20-40% positive with a relative intensity of one. Mutations of the *ras*, p53, and DCC genes are three common occurrences during the onset and progression of colon cancer. Further molecular characterization of this premalignant cell line will be an invaluable model for the study of genetic events involved in UC-associated colon cancer.

**V-1033** Development And Characterization Of Human Normal And Cancerous Stomach Epithelial Cell Lines. L.A. MANZANO, G.C. Balch, J.S. Stauffer, and M.P. Moyer. Department of Surgery, University of Texas Health Science Center at San Antonio, TX 78284.

The incidence and occurrence of gastric cancer has generated a significant amount of clinical and basic research even though the factors in the development of the disease are still not fully understood. We feel that we can significantly contribute to the elucidation of this information with two recently established gastric epithelial cell lines. The first line is of normal human stomach mucosal origin (NHSM57) while the second was derived from a human stomach tumor (HST64). Both cell lines, now at Passage 8, were processed mechanically and have been continuously cultured as monolayers for over a year. Soft agar studies have demonstrated that NHSM57 does not form colonies in soft agar while HST64 does. Immunocytochemical staining has shown both cell lines to be of epithelial origin, and in fact demonstrate a positive reaction with carcinogenic embryonic antigen (CEA) for HST64. Immunocytochemical staining of NHSM57 for the expression of oncogene and/or tumor suppressor gene products has demonstrated positive reactions for mutant p53 and MDM2 while all other antibodies were negative. As we expand our molecular characterization of these lines, we strongly believe that the lines will be of great benefit as an in vitro human cell culture model that will further expand the scope of knowledge relating to progression of gastric cancer.

**V-1034** Development And Characterization Of Two Novel Human Colon Polyp (COP) Cell Lines With Differing Degrees Of Tumorigenicity. J.S. STAUFFER, L.A. Manzano, G.C. Balch, R.L. Merriman\*, L.L. Tanzer\*, and M.P. Moyer. Department of Surgery, University of Texas Health Science Center at San Antonio, TX 78284; \*Eli Lilly Co., Indianapolis, IN 46285.

Attempts have been made to develop a colon polyp cell line for many years for use as an in vitro model for colon carcinogenesis. We have developed two cell lines that promise to fill this gap. COP24 and COP29 are polyp lines derived from patients undergoing operative procedures. They were processed mechanically and placed in culture. COP24 is currently at Passage 8 and COP29 at Passage 7. Both have shown consistent growth characteristics for almost a year. Immunocytochemistry has proven the epithelial origin of these cells. Soft agar assays show that COP29 forms colonies in soft agar and COP24 does not. Tumorigenesis studies demonstrate that COP24 forms tumors in nude mice and COP29 does

not. Immunocytochemical tumor markers show the following:

Cell	mutant p53	ras	DCC	APC	MDM-2
COP24	+80%	+10%	+20	+70%	+40%
COP29	+80%	+20%	-	+10%	+20%

COP24 expresses DCC, APC, and MDM-2 which is consistent with current thinking about the progression of polyps to colon cancer. Expression of the ras gene and mutant p53 may explain why these cells have become immortalized. Both cell lines promise to add a potent in vitro weapon for further elucidation of the normal to tumor progression of colon cancer.

**V-1035** Bacterial L-Forms Are Omnipresent In "Sterile" Tissue Culture Sera. J.W. HAYCOCK<sup>1</sup>, K.E. Joho<sup>1</sup>, J.C. Galyon<sup>1</sup>, and R.L. Moses<sup>2</sup>. Departments of <sup>1</sup>Biochemistry & Molecular Biology and <sup>2</sup>Anatomy, Louisiana State University Medical Center, New Orleans, LA 70119.

The presence of viable, L-form (cell wall-deficient) bacteria was inferred in each of over 60 animal sera from several different commercial suppliers. The appearance of L-form growth was slow and did not exhibit the "bloom" associated with normal bacteria. Thus, with frequent medium changes, such contamination is easily overlooked and/or discounted as "cell debris," which may account for the dearth of such reports. After incubation of sera (aliquots or unopened bottles; 4-37° C; 0-4 wks), multiple morphological forms could be observed by light microscopy in each sample. L-form growth occurred 1) in the absence of cultured mammalian cells; 2) in neat sera or serum-supplemented media, but not in media alone; 3) in sera supplied prefiltered (0.2 - 0.04 µm); and 4) in heat-inactivated (2 h, 60° C) and refiltered (0.2 - 0.1 µm) sera. Several sera, as well as a contaminated cell culture, tested negative for mycoplasma in two clinical labs. TEM profiles resembling those previously reported for L-forms were found in sera as well as *within* cells from several established cell lines. Extensive antibiotic trials and several alternative treatments (α-irradiation, β-propiolactone, ethylene oxide, Hoechst 33258, thimerosal) failed to prevent the appearance of L-form growth. However, ultrafiltration (≤300,000 MW cut-off) appeared to eliminate L-form growth, thus providing a potentially feasible technique for commercial preparation of L-form-deficient sera. By contrast, purging established cell lines may prove to be exceedingly difficult. Recently, we have amplified several nucleotide sequences from DNA extracts of serum fractions using broad-range eubacterial 16S rDNA primers. These partial sequences should enable *in situ* hybridization studies, and complete 16S rDNA sequences should allow identification of the organisms.

**V-1036** Isolation And Characterization Of Functional Dendritic Cells In Rat Choroid. A. CHOUDHURY<sup>1</sup>, V.A. Pakalnis<sup>1,2</sup>, and W.E. Bowers<sup>1,2</sup>. Departments of Microbiology & Immunology<sup>1</sup> and Ophthalmology<sup>2</sup>, University of South Carolina School of Medicine, Columbia, SC 29208.

Dendritic cells (DC) are potent stimulators of T-cell mediated responses. This study, for the first time, describes the isolation and culture of functional DC from the posterior segment of rat eye. Cells isolated from the posterior segment by enzymatic dissociation could stimulate T-cells polyclonally activated by mild sodium periodate treatment as well as allogeneic T-cells in Mixed Leukocyte Cultures. Separation of the posterior segment cells in a discontinuous gradient revealed that the functional activity was almost entirely associated with low density cells, a property characteristic of DC. Using immunofluorescent staining with anti-DC antibodies and confocal microscopy we could detect cells of DC morphology in cryostat sections as well as cultured cells from rat posterior segment. DC could be further purified by immunomagnetic separation using a panel of anti-DC antibodies that we produced. Immunomagnetically purified DC were cultured with a variety of cytokines and metabolic modulators to study the effect of these agents on DC function. IL-1β and granulocyte-macrophage colony stimulating factor (GM-CSF) caused a significant increase in the lymphostimulatory ability of DC. The results suggest that regulation of DC function in the posterior segment may have significant implications in uveitis and other inflammatory diseases of the eye.

**V-1037** Production And Characterization Of A Monoclonal Antibody Specific To *Mycobacterium tuberculosis*. JYOTIKA KAPUR, S.K. Gupta\*, and G.P. Talwar\*. Department of Veterinary Immunology, Punjab Agricultural University, Ludhiana-141004, India; \*National Institute of Immunology, New Delhi, India.

The production and characterization of a murine monoclonal antibody P6, reactive to *Mycobacterium tuberculosis* is described. This monoclonal is unique in its absence of reactivity with BCG and nonvirulent strain of *M. tuberculosis*, i.e. H<sub>37</sub>Ra. This antibody, an I<sub>g</sub>G1/k type, was tested against sonic extracts obtained from 27 mycobacteria and 39 bacteria, by an enzyme-linked immunosorbent assay. It was devoid of reactivity with all these bacteria except for a low reaction with *Nocardia asteroides* (11.8%). Apparently, it was highly specific for the virulent form, the H<sub>37</sub>Rv. It recognized intact as well as sonicated bacteria. Various pathogenic strains of *M. tuberculosis* collected from different geographical locations, showed a range of reactivity with this monoclonal. Epitope recognized by this antibody is of repetitive type, present on 45 and 96 kDa proteins of H<sub>37</sub>Rv. Potential of this monoclonal in the development of diagnostics would be discussed.

- V-1038** Role of Interferon On Influenza-Induced Genotoxicity. M.V. RAMANA and Gita Sharma. Department of Microbiology, Osmania University, Hyderabad-500 007, India.

We have demonstrated that influenza virus is a biological mutagen, as it induces chromosomal aberrations in spermatocytes leading to sperm head abnormalities, sterility and dominant lethality and also transmits aberrations to the  $F_1$  progeny. Our data also indicates that the administration of inactivated influenza virus also causes the effects that the infective virus is capable of. One of the cell-virus interactions is the induction of interferon leading to an antiviral effect. Hence, we have studied the role of interferon on the genetic effects due to influenza virus infection. Details of these findings and its implications will be presented.

- V-1039** An Efficient Method For Routine Epstein-Barr Virus Immortalization Of Human B Lymphocytes. F.E. WALL, R.D. Henkel, M.P. Stern, and M.P. Moyer. Center for Human Cell Biotechnology, Department of Surgery, The University of Texas Health Science Center at San Antonio, TX 78284.

A variety of methods exist for the immortalization of B lymphocytes by Epstein-Barr virus due to the simplicity of such techniques to establish continuous cell lines which can be maintained indefinitely and used for studies that require large amounts of stable genomic DNA. Although these methods frequently result in transformation, the variability in success within a rapid time frame reduces confidence in use of method selection when there are large numbers of pedigreed donors (e.g., for genetic studies), relatively small numbers of collected cells, and the need to bank large numbers of cells as quickly as possible. Because of these requirements and the precious nature of these resources, we undertook this study to provide a routine method to facilitate EBV-induced B lymphocyte immortalization. Two methods were compared: Method 1, which is a general composite of standard methods and Method 2, in which a composite method includes supplementation of the culture growth medium with conditioned medium from an EBV-transformed B cell line. An incubation period in Epstein-Barr virus and the use of conditioned media improved immortalization efficiency from 86% to 98% and decreased the time (usually weeks) from culture initiation to cryopreservation. Conditioned medium eliminated the need to grow up, maintain, and irradiate flasks of feeder cells while still providing growth and other factors for newly initiated cultures. This medium also aided in removing cells from cryogenic storage by increasing cell recovery and viable yield. The resulting cell bank was used to produce DNA for genetic studies focusing on the genes involved in non-insulin dependent diabetes mellitus. (Supported from NIH Grant #DK42273 and STRC Grant #A-200.)

- V-1040** Effect Of Serum-Free Media And Extracellular Matrix On The Growth And Gene Expression Of OR-HEPA  $B_3$  Cells. M. VEGA, N. Cordero, and M. Morales. University of Puerto Rico, Biology Department, San Juan, Puerto Rico 00931.

To avoid the shown inhibitory effects of serum on gene expression, hormonally defined media has been developed in our lab to study the growth and expression of tissue-specific genes in a mouse hepatoma cell line, OR-Hepa  $B_3$ . We have found that cells grown in media supplemented with low concentrations of FBS (2% - 0.5%) show similar growth than in 5% FBS. Analysis of secreted and intracellular proteins as well as of RNA indicate no differences under these conditions. This allows the treatment of cells grown in 0.5% FBS with extracellular matrix (ECM) components, since it offers a system essentially serum-free. We have found that Waymouth medium supplemented with type I collagen 10  $\mu\text{g}/\text{cm}^2$ , fibronectin 2  $\mu\text{g}/\text{cm}^2$ , insulin 5  $\mu\text{g}/\text{ml}$ , transferrin 5  $\mu\text{g}/\text{ml}$ , selenium 0.005 ng/ml and linoleic acid 10  $\mu\text{g}/\text{ml}$  maintains a growth comparable to the one observed with Waymouth supplemented with 0.5% FBS for more than 72 h. The electrophoretic pattern of secretory products under this condition shows differences when compared with cells under serum treatment; nevertheless, the intracellular pattern is almost the same. Western blot analysis shows the secretion of albumin and transferrin. Studies using defined media and ECM in combination with other gene regulators is currently under progress. (Supported by MBRS/NIH.)

- V-1041** Effects Of Interstitial ECM On The Viability Of Human Neutrophils *In Vitro*. E.J. ROEMER, K.J. Stanton, and S.R. Simon. Pathology Department, SUNY at Stony Brook, Stony Brook, NY 11794-8691.

Leukocytes migrate to and through the interstitial stroma and degrade extracellular matrix (ECM) components in both acute and chronic inflammation. To evaluate the pro and anti-inflammatory potential of various agents we have developed *in vitro* assays of leukocyte-mediated degradation of interstitial ECM using a native interstitial matrix produced by cultured R22 rat heart smooth muscle cells. Human neutrophils (PMN) were used as sources of degradative activity. Viability and activation of these cells *in vitro* is of critical importance in these assays. PMN were added to uncoated polystyrene wells and to ECM-containing wells in microplates and were activated with concentrations of phorbol ester (PMA), an especially effective stimulus, from 0.10 nM to 10 nM. Viability and respiratory burst activity were assessed respectively by Trypan blue exclusion and by reduction of a tetrazolium dye (MTT) to its formazan. PMN adhered to the ECM experience increasing respiratory burst activity in the presence of increasing PMA concentrations up to 0.25 nM without loss of viability over 24 hours. At 0.5 nM PMA, viability of ECM-adherent PMN is reduced to six hours, and most cells are lysed by 24 hours. PMA concentrations  $\geq 1.0$  nM kill and lyse PMN on the ECM within 4 hours. Even unstimulated PMN have reduced viability on polystyrene, and at PMA

concentrations around 0.25 nM (at which ECM-adherent PMN are viable for 24 hours) PMN on uncoated polystyrene wells are nonviable and extensively lysed by 4-6 hours. These results emphasize the importance of the interstitial microenvironment for determining PMN viability, especially after exposure to activating stimuli.

**V-1042** Differentiation Of Stem Cells From The Midgut Of The Insect, *Manduca sexta*, Occurs *In Vitro* In The Presence Of A Factor From Mature Larval Midgut Cells. S. Sadrud-Din<sup>1,2</sup>, R.S. Hakim<sup>1</sup>, M.J. LOEB<sup>2</sup>. <sup>1</sup>Howard University, Washington, DC; <sup>2</sup>INHL, U.S. Department of Agriculture, Beltsville, MD 20705.

Midgut epithelium from molting fourth instar larvae of the lepidopteran, *Manduca sexta*, has been cultured in modified Grace's medium containing 20 hydroxyecdysone and co-cultured pupal fat body. In this mixed cell culture, stem cells proliferated and differentiated to freely suspended goblet and columnar cells. Cultures of isolated stem cells were prepared by gentle agitation of midgut fragments in medium; only the loosely associated stem cells were released from the tissue. Stem cells incubated in modified medium with fat body proliferated but did not differentiate. However, addition of conditioned medium from a mixed cell culture did induce differentiation. The differentiation factor appears to be a small peptide of less than 10 kDa.

**V-1043** An Organ Culture Model Of The Embryonic Murine Ear. D.S. HOFFMAN<sup>1</sup>, P. Bringas<sup>2</sup>, and H.C. Slavkin<sup>2</sup>. <sup>1</sup>Department of Otolaryngology, Head and Neck Surgery, University of Southern California School of Medicine, 1200 N. State Street, Los Angeles, CA 90033; <sup>2</sup>The Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, CA 90007.

Embryonic development of the mammalian ear is a complex multistep process involving sequential interactions between the neuroectoderm, the first and second branchial arches, and subjacent mesenchyme. To study the role specific growth factors and transcription factors play in this process, our first step has been to establish an organ culture system of the embryonic murine ear. Microdissection of 38 to 42 somite mouse embryos generated explants consisting of the first and second branchial arch, the otocyst, and the adjacent neural tube. The growth of such explants in serum- and growth factor-free media, as well as in media supplemented by serum, NGF, or TGF beta-3, was studied by H&E staining, Alcian blue whole mount staining, and SEM. An assay for LDH released into the media was used as an objective measure of cell death. Although explant viability and some aspects of histogenesis are improved by supplementation, several features of ear development are recapitulated *in vitro* even without supplementation. Explants grown in growth factor-free media exhibit recognizable pinnae, a semicircular canal, a short cochlear duct, and a chondrified otic capsule. Thus, paracrine and/or autocrine factors mediate many aspects of ear morphogenesis. (This work was supported in part by the

Center for Craniofacial Molecular Biology, under NIH grant DE-09165.)

**V-1044** Properties Of The Hepatix C3A Human Hepatoblastoma Cells Cultured In A Hollow Fiber Bioreactor. ANDREA SPIERING, Daer He, Mary Harrison, Yuet Wan Lo, Avi Rotem, Normal L. Sussman, and James H. Kelly. Hepatix Inc., Houston, TX 77082.

Hepatix C3A cells are being used as the foundation for an extracorporeal liver assist device (ELAD) with the intention of supplying sufficient liver function to support patients in fulminant hepatic failure. We have used a series of *in vivo* and *in vitro* studies to assess the ability of the ELAD to carry out broad based liver-specific metabolism. Approximately  $1 \times 10^9$  cells are inoculated into a cellulose acetate hollow fiber device with 1.7 m<sup>2</sup> surface area. Growth of the device is monitored using glucose consumption and albumin production. At release, each device consumes over 10 g of glucose/day and synthesizes approximately 2 g of human albumin/day, 17% of the adult liver. Galactose uptake is used in a clinical setting to monitor total viable hepatocyte mass. *In vitro*, galactose uptake by the ELAD is linear and has a rate of 120 μM/min, about 25% of the rate of normal liver. *In vivo*, a single, mature device can supply about 15% of the normal galactose elimination capacity (GEC) to an anhepatic patient. The device is capable of clearing 90 g lactate per day in a patient with lactic acidosis. Under normal circumstances, the liver clears 120 g of lactate per day. The device metabolizes lidocaine to its major deethylation product, MegX, which is detected within 30 min of injection. The rate of lidocaine clearance is 17% of the normal liver. Each of these estimates is consistent with a single ELAD representing 15-20% of the normal liver mass, the minimum required for realistic therapeutic capability. Rates of clearance are about 20% of normal due to the less favorable geometry of the bioreactor compared with the natural organ.

**V-1045** Selective Expression Of Prostaglandin H Synthase-1 And -2 During Differentiation Of Rat Tracheal Epithelial Cells. E.M. Hill<sup>1</sup>, T. BADER<sup>2</sup>, P. Nettesheim<sup>2</sup>, and T.E. Eling<sup>1</sup>. <sup>1</sup>Laboratory of Molecular Biology and <sup>2</sup>Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Primary rat tracheal epithelial (RTE) cells cultured at an air-liquid interface can differentiate into one of two phenotypes. In the presence of retinoic acid (RA), RTE cultures differentiate into a mucociliary phenotype secreting large quantities of mucin glycoproteins and closely resembling the morphology of the tracheal epithelium *in vivo*. RA-depleted cultures differentiate into a stratified squamous epithelium with a cornified apical layer. After 10-12 days of culture both phenotypes appeared terminally differentiated with maximum expression of mucin or keratin markers and a reduction in cell proliferation. At this time both phenotypes showed prostaglandin H synthase (PHS) activity as measured by the production

of PGE<sub>2</sub> from both endogenous and exogenously supplied substrate. Expression of PHS-1 and -2 isoforms during differentiation of the two phenotypes was investigated. When RTE cells grew in the presence of RA, levels of PHS-1 mRNA decreased during differentiation whereas PHS-2 mRNA levels increased 4-fold. This increase was accompanied by a similar increase in PHS-2 protein levels and a 10-fold increase in PGE<sub>2</sub> production during differentiation to the mucociliary phenotype. Time course studies during mucociliary differentiation showed a close correlation between the production of PGE<sub>2</sub> and mucin, suggesting a role for this prostanoid in regulation of mucin secretion. In RA-deficient cultures both PHS-1 and -2 mRNAs were maintained at a basal level consistent with a low level of PGE<sub>2</sub> production during differentiation to the squamous phenotype. These studies show that the two PHS isoforms are selectively expressed during differentiation of RTE cells to either mucociliary or squamous cells.

**V-1046** Regulation Of Growth And Secretory Differentiation In Cultured, Normal, Early Passaged Human Bronchial Epithelial (HBE) Cells. T GRAY, T. Bader, and P. Nettesheim. Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Our laboratory is interested in studying the regulation of growth and differentiation of airway epithelial cells. Recently we described an organotypic culture system in which rat tracheal epithelial cells seeded at  $\sim 2 \times 10^4$  cell/cm<sup>2</sup> undergo rapid growth becoming confluent in 6-8 days, and ultimately produce a mucociliary epithelium upon reaching cell densities of  $1-2 \times 10^6$  cells/cm<sup>2</sup>. Using a similar culture system we have found that human bronchial epithelial cells when seeded at  $1 \times 10^4$  cell/cm<sup>2</sup> never achieve confluency, only reaching cell densities of  $1-2 \times 10^5$  cells/cm<sup>2</sup>. Histological evidence for secretory cell differentiation in these HBE cell cultures is not convincing yet we have detected by slot blot analysis using the monoclonal antibody 17Q2 (gift from J. St. George) the presence of a mucin-like glycoprotein in the apical fluid of the culture. We have observed and others have reported (M. Yamaya et al., *Am. J. Physiol.* 262:L713-L724, 1992) that cultures seeded with  $1 \times 10^6$  cells/cm<sup>2</sup> form a confluent cell layer in 6-10 days and undergo secretory cell differentiation evidenced by the presence of cells containing secretory granules and the production of detectable mucin-like glycoprotein. The goal of this study will be to define culture conditions in which HBE cells grow to cell high density which we hypothesize to be a prerequisite in order for mucociliary differentiation to occur. The role of seeding cell density, the presence of retinoids, the role of TGF- $\beta$  and the establishment of air-liquid interface on growth and induction of secretory and ciliated cell differentiation will be investigated.

**V-1047** Regulation Of Mucin Expression By Retinoic Acid (RA) And Culture Conditions In Rat Tracheal Epithelial (RTE) Cells. V.B. GODFREY<sup>1</sup>, K. Guzman<sup>1</sup>, C.B. Basbaum<sup>2</sup>, P. Nettesheim<sup>1</sup>, and S.H. Randell<sup>1</sup>. <sup>1</sup>Laboratory of Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC 27709; <sup>2</sup>University of California, San Francisco, CA.

Goblet cell hypertrophy, hyperplasia, and mucus hypersecretion are pathognomonic of several respiratory tract diseases. Retinoids and culture conditions play a vital role in supporting mucociliary differentiation of airway epithelial cells but it remains unclear whether their effect on mucin production is a consequence of fostering secretory cell differentiation or if they act directly on mucin gene expression. The purpose of our studies was to examine the role of RA and culture conditions on expression of a mucin gene and production of mucin glycoproteins. Primary RTE cells were cultured on plastic or collagen-gel coated Transwell-COL inserts in the presence or absence of  $5 \times 10^{-8}$  M RA (Kaartinen et al., *In Vitro*, 1993). RAM7S cDNA (Tsuda et al., *BBRC*, 1993) was used as a probe for northern analysis. RTE11, a monoclonal antibody specific for rat mucin carbohydrate moieties (Shimizu et al., *Exp. Lung Res.* 1992), was used in a slot blot assay to measure the amount of mucin glycoprotein in conditioned media of plastic dishes and in apical washings of the insert cultures. When studied on Day 14 of culture, RA induced RAM7S mRNA in RTE cells on plastic or inserts. However, mucin glycoprotein secretion was only detected when RTE cells were maintained on inserts in the presence of RA. Further studies are needed to evaluate other time points, and to elucidate whether the collagen gel or porous membrane support were critical for mucin glycoprotein production. Our data indicates that RA is necessary but not sufficient for mucin glycoprotein production which is likely regulated by a complex pattern of both transcriptional and post-transcriptional events.

**V-1048** Flow Cytometric Analysis Of Mouse Bone Marrow. G.D. KALMAZ. Division of Hematology, University of Texas Medical Branch, Galveston, TX 77555-0567.

The hemopoietic system is composed of cells of several different lineages at various stages of differentiation. In this study, mouse bone marrow was analyzed using 3,3'-dihexyloxycarbocyanine iodide (DiOC<sub>6</sub>[3]). Bone marrow was collected from the femurs of BALB-C male mice. Smears were prepared to compare findings with flow cytometry results, and 500 cells were analyzed manually for each smear. Cytospin preparations obtained after sorting by flow cytometry were also examined manually to see whether the correct population of cells was isolated. Marrow was gently flushed into PBS, and single-cell suspensions were prepared and centrifuged at 300 g for 5 min. Marrow pellets were then resuspended in PBS with 1% dextrose. To determine the total nucleated count, samples from unstained bone marrow suspensions were analyzed with a Coulter counter (Coulter Corp., Hialeah, FL). The remaining suspensions were stained with DiOC<sub>6</sub>(3) for 30 min at 37° C in

the dark on ice and were brought to room temperature immediately before flow cytometric analysis in an Epics C flow cytometer (Coulter) using an excitation wavelength of 488 nm from an argon laser. Gates were set to exclude debris. Two-parameter histograms—log integral green fluorescence versus forward angle light scatter—were generated to gate on proliferating myelocytes, lymphocytes, proliferating erythrocytes, and mature erythrocytes; 10,000 cells were analyzed from each sample. A paired *t* test was used to evaluate the data. Results showed that analysis of mouse bone marrow by flow cytometry using a conventional reagent is a simplified approach and offers distinct advantages over current manual methods for evaluating bone marrow specimens.

**V-1049** Pyridoxine HCl: An Equivalent Form Of Vitamin B-6 For Dulbecco's Modified Eagle's (DME) Cell Culture Medium. B. ALDERETE, L. Balog, J. Doak, K. Etchberger, R. Festen, and J. Keathely. JRH Biosciences, 13804 W. 107th Street, Lenexa, KS 66215.

Providing sufficient and necessary nutrients in cell culture media is a constant challenge to manufacturers and end users. Adequate shelf-life of ready-to-use liquid media is also an important consideration. In an effort to extend the shelf-life of liquid Dulbecco's modified Eagle's (DME) media, we have examined the effects of substituting the standard form of Vitamin B6, pyridoxal HCl (PL), with pyridoxine HCl (PN) on cell growth performance and the formation of insolubles. Preliminary results of cell growth assays show equivalence of pyridoxine HCl containing DME as compared to the standard pyridoxal HCl formulation. Multiple passage of 3T3 cells in PN-DME grew to 86-125% of the PL formulated DME media. Four day proliferation assays of 3T3 cells measured by the cellular conversion of tetrazolium salts yield similar absorbances for the two groups, PL = 0.13  $A_{550}$  units vs. PN = 0.14  $A_{550}$  units. Plating assays of A549 cells show similar data: PN-DME grew 83-125% of the number of colonies as compared to standard PL formulated DME. Upon storage of liquid media at 2° C - 8° C for 9 months, 570 of 630 liter containers (90.4%) of PL-DME developed visible precipitation, but 0 of 765 liter containers of PN-DME precipitated under the same conditions. Also, aqueous PL solutions are sensitive to heat, but PN may be heated without decomposition. These data demonstrate 1) that pyridoxine HCl in DME provides equivalent cell growth as compared to the pyridoxal HCl, and 2) the pyridoxine HCl supplemented liquid DME is less likely to develop precipitation upon prolonged cold storage.

**V-1050** Growth Of Myogenic Cells In Media Supplemented With Peptide-Bound Essential Amino Acids. Y.L. PAN, K.E. Webb, Jr., and P.K. Bender. Departments of Animal & Poultry Sciences and Biochemistry & Nutrition, Virginia Tech, Blacksburg, VA 24061.

When expressed as a percentage of the total plasma amino acid pool, peptide-bound amino acids account for 10% in man, 50-80% in ruminants, 10-50% in rats. The

functions of most of these circulating peptides remain unknown. Our objective was to investigate the growth of cultured myogenic cells in media supplemented with peptide-derived essential amino acids. The basal media were either lysine-free or methionine-free Dulbecco's modified Eagle's medium supplemented with 6% de-salted fetal bovine serum that was prepared by passing serum through a Sephadex G-25 column to remove free and peptide-bound amino acids. In lysine-free basal medium supplemented with di- to septi-peptides containing one or two lysyl residues, protein accretion in  $C_2C_{12}$  mouse myogenic cells ranged from 20 to 100% of that with equimolar amount of free lysine. Protein accretion was independent of the number of amino acid residues in the peptides. Primary cultures of ovine skeletal muscle incubated with methionine-free basal medium accumulated 15-20% more protein and DNA in the presence of methionylmethionine and methionylglycine than in the presence of glycylmethionine. In contrast, the cytoplasmic fraction of ovine skeletal muscle hydrolyzed glycylmethionine more rapidly than methionylmethionine and methionylglycine. This showed that cytoplasmic peptidase activity was not the limiting factor for the utilization of these three dipeptides by cultured ovine myogenic cells. Additional studies are required to examine the roles of plasma membrane-bound peptidase activity and peptide transporters in the utilization of peptides by myogenic cells.

**V-1051** The Effect Of Secretory Factors Of A Hepatoma Cell Line As Growth Regulators. Y. ORTIZ, N. Cordero, and M. Morales. University of Puerto Rico, Biology Department, San Juan, Puerto Rico 00931.

Cellular growth is controlled by extracellular signals, generally provided *in vitro* by animal sera added to culture medium; nevertheless, proliferation of transformed cells has been shown to occur in serum-free media. This indicates the capacity of these cells to regulate their growth by secreting autocrine growth factors. In our lab we have isolated secretory products (PSB<sub>3</sub>) from a hepatoma cell line, OR-HEPA B<sub>3</sub>, to study its effects as growth promoters. For the preparation of these factors, medium was recollected after two consecutive periods of 48 h serum-free cultures, dialyzed and concentrated. Supplementation of Waymouth medium with 100 µg/ml of PSB<sub>3</sub> was able to maintain OR-HEPA B<sub>3</sub> cells growing at 43-90% of the growth obtained in 5% FBS. PSB<sub>3</sub> also maintains the growth of the hepatoma cell line BWIC3 and of the fibroblast-like line L929 at 53% and 75%, respectively, of the growth obtained in 5% serum, but it was not able to maintain the growth of the human sarcoma line WEHI; on the contrary, a dramatic growth inhibition was evident even in the presence of 5% serum. Analysis of the secretory protein pattern of the hepatoma cell lines under 5% FBS shows no major changes by the PSB<sub>3</sub> treatment. Since transferrin is one of the proteins secreted by this hepatoma cell line, studies are currently addressed to determine if the growth effects of PSB<sub>3</sub> are due to the mitogenic properties of this protein. (Supported by MBRS/NIH.)

- V-1052** Utilization Of Peptide-Bound Methionine For The Synthesis Of Secreted Proteins By Cultured Bovine Mammary Epithelial Cells. S. WANG, K.E. Webb, Jr., and R.M. Akers. Departments of Animal and Poultry Science and Dairy Science, Virginia Tech, Blacksburg, VA 24061.

A bovine mammary epithelial cell line (MAC-T) was used to study the ability of 17 methionine-containing di- and tripeptides to substitute for free methionine in the synthesis of secreted proteins. Cells were plated at 100,000 cells/well (24-well plate) and allowed to grow/differentiate at 37° C in a humidified atmosphere of 95% air/5% CO<sub>2</sub> for 3 or 8 d. The cells were then incubated in methionine-free Dulbecco's Modified Eagle's Medium supplemented with insulin, prolactin, dexamethasone, <sup>3</sup>H-leucine, and methionine or one of the methionine-containing peptides for 3, 6, or 24 h. The ability of methionine substrates to promote incorporation of <sup>3</sup>H-leucine into secreted and cell proteins was quantified. Effects on cell proliferation were determined by measuring DNA content. Cells were able to utilize methionine from all peptides tested. Ability of the peptides to promote incorporation of <sup>3</sup>H-leucine into secreted proteins varied with experimental conditions. Generally, difference among peptides and between peptide and free methionine were greater at 3 and 6 h incubation compared with 24 h incubation. For cells allowed to grow/differentiate for 3 or 8 d, incorporation of <sup>3</sup>H-leucine promoted by peptides after 3 h incubation ranged from 67 to 85% and 86 to 110% of the incorporation promoted by free methionine, respectively. Cell proliferation was not influenced by peptides. Results suggest that di- and tripeptide-bound methionine can serve as a source of methionine for the synthesis of secreted proteins by MAC-T cells. The cells with longer growth/differentiation periods (8 vs. 3 d) appear to have a greater ability to utilize the peptides.

- V-1053** Three-Dimensional Culture Of Bovine Chondrocytes In Rotating-Wall Vessels. Tracey L. Prewett<sup>1</sup> and Thomas J. Goodwin<sup>2</sup>. <sup>1</sup>KRUG Life Sciences, Houston, TX 77058; <sup>2</sup>NASA/Johnson Space Center, Houston, TX.

In this study, the Rotating-Wall Vessel (RWV) is used to culture chondrocytes in the presence of Vitamin C for 40-45 days to observe the effect of low-shear and quiescent culture conditions allowing three-dimensional freedom for growth, differentiation, and ECM formation. Previous studies demonstrated that culture conditions influence the phenotype of the chondrocytes. Collagen and agarose gel systems are used extensively in the routine culture of the chondrocyte hypertrophic phenotype present during endochondral bone formation or chondrocyte differentiation. Extracellular matrix (ECM) influences differentiation of chondrocytes. Synthesis of Type X Collagen by hypertrophic chondrocytes is modulated by ECM molecules including collagen, proteoglycans, fibronectin, and other agents such as alkaline phosphatase, Vitamin D, and Vitamin C. Bovine

chondrocytes were freshly isolated from bovine cartilage and placed into the RWV with Cytodex-3 microcarriers. Non-adherent petri dishes were initiated with microcarriers as representative of standard culture conditions. In the RWV, large three-dimensional aggregates (5-7 mm) were formed in suspension. In the RWV a large sheet of matrix adhered to the oxygenator core and vessel endcaps. Petri dish culture resulted in the formation of sheets of chondrocytes with no matrix production. Immunocytochemical analysis on histologic sections of tissue obtained from the RWV and the petri dish controls demonstrated significantly increased vimentin signal in the RWV material while chondroitin-4 sulfate, chondroitin-6 sulfate, and fibronectin were comparable in both settings. These results indicate that the unique conditions provided by the RWV afford access to cellular processes that signify the initiation of differentiation as well as production of normal matrix material.

- V-1054** Isolation, Purification And Quantitation Of Mast Cells From Rat Uterus. V. CHOPRA and R.E. Garfield. Reproductive Science Division, Department of Obstetrics and Gynecology, UTMB, Galveston, TX 77555-0587.

Mast cells were isolated from rat uterus using enzymatic digestion and isotonic percoll gradient centrifugation on Day 15-18 of gestation; during delivery (Day 22) and one day postpartum. A total of 5 x 10<sup>7</sup> cells/g of tissue were harvested on the day of delivery; in contrast to 1 x 10<sup>7</sup> cells/g of tissue on Day 18 and 1.5 x 10<sup>7</sup> cells/g on one day postpartum (N = 6). Sixty-one percent of cells were alcian blue positive (AB+) on the day of delivery in contrast to 34% AB+ cells during gestation and 14% AB+ cells during postpartum period (viability = 68%). There was a three-fold increase in the β-Naphthyl Esterase positive cells during delivery (75%) as compared to gestation and postpartum period. This was associated with an increase in monocytes and lymphocytes when stained by differential staining. There was no change in the number of basophils, neutrophils, and eosinophils. On further purification of cells using percoll gradients, 75% of cells were AB+ at 50/60 interface (lighter density cells), and 62% of cells were AB+ at 60/80 interface (heavier density cells) during delivery on Day 22 of pregnancy. In contrast, only 32% of cells were AB+ during gestation; 37% of cells were AB+ one day postpartum at 50/60 interface; 38% of cells were AB+ during gestation; and 42% of cells were AB+ one day postpartum at 60/80 interface. Thus, there was a decrease in the percentage of viable AB+ cells during gestation and postpartum period. These studies indicate that there is a relative increase in the percentage of viable AB+ mast cells, monocytes, and lymphocytes during delivery, on the basis of their density and differential staining. These two populations could be further studied to extrapolate on the role of mast cells on uterine contractility.

- V-1055** A Method For The Culture Of Proliferating Sheets Of Sensory Cell Progenitors Isolated From The Chick's Cochlea. J.E. FINLEY and J.T. Corwin. Departments of Otolaryngology and Neuroscience, University of Virginia, Charlottesville, VA 22908.

Organ cultures of the avian ear have been used in investigations aimed at the eventual development of treatments for hearing loss and balance disorders, but they have inherent limitations. Here we report a method for the culture of isolated sheets of supporting cells of auditory epithelia which are the progenitors of regenerated sensory hair cells. In our most successful method, cochleae are dissected from 1- to 20-day-old chicks, placed in Ca/Mg-free HBSS, and the tegmentum vasculosum is dissected away. After a 60 sec incubation in protease type VIII (Sigma) at 34 µg/ml, the tectorial membrane is removed. The sensory epithelium is lifted off the basilar membrane and cut into segments. The segments adhere to culture wells in 2-3 days in M-199 + 20% FBS at 5% CO<sub>2</sub>. Hair cells begin extruding from the sheets in the first 24 h. By 72 h all hair cells have been ejected, and the supporting cells have begun to proliferate. There is little or no fibroblast contamination. Anticytokeratin staining is strong and typical for epithelial cells. Anti-vimentin staining is distinct from the staining pattern in control fibroblasts. The growing colonies of supporting cells expand, label with BrdU, and remain healthy for up to 4 weeks as indicated by LIVE/DEAD™ assay. These cultures of the isolated progenitors of auditory receptor cells appear to provide useful models in the form of relatively homogeneous colonies that are easily grown in multiwell plates for investigations of the mechanisms of hair cell regeneration. (Funded by the NIDCD.)

- V-1056** The Culture Of Chick Embryo Ovary As A Model In Experimental Tumorigenesis. R. AVILA, M. Samar, S. de Fabro, and R. Ferraris. Department of Histology and Pathology, School of Medicine, National University of Cordoba, CONICOR, CONICET. Cordoba (5000) Argentina.

Preliminary studies using ovarian cultures of chick embryos with the addition of 17-β-estradiol, testosterone propionate, progesterone, FSH, LH or hCG, allowed to demonstrate, by means of cytochemical and ultrastructural analyses, that membrane differentiations and muco-substances had a greater development induced by estradiol, testosterone or progesterone in the left ovary, which is the one that matures, as compared with the right one, that involutes. FSH acted similarly on both ovaries, producing cell regression, whereas LH and hCG increased membrane differentiations and mucosubstances. These results show that cell surfaces would be modulated by the action of these hormones during ovarian differentiation. Considering that embryo cells have a behavior similar to that of some tumor cells, the effects of tamoxifen, vitamin A and retinoic acid, employed in antitumoral therapy, will be analyzed on cultured ovaries. The cultured ovary represents an adequate model for the study, not only of the processes

of cellular differentiation and regression, dependent of hormones' action, but also to study cell membranes and their differentiations under the effects of tamoxifen, vitamin A, and retinoic acid.

- V-1057** Prohibitin Expression In Immortalized Cell Populations. R.T. DELL'ORCO, X-T. Liu, E.R. Jupe, J.L. Kiehlbauch, and J.K. McClung. Noble Center for Biomedical Research, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Prohibitin is an evolutionarily conserved gene that possesses antiproliferative activity. It is postulated to be a tumor suppressor gene whose expression, when lost, contributes to the immortalization of cells from one or more of the four complementation groups proposed by Pereira-Smith. The presence and expression of the prohibitin gene in representatives from each of the complementation groups were compared to normal populations by Southern, Northern, and Western analysis. Initial results show that the gene is present in all cell lines tested; however, it is polymorphic and appears to be present at a higher gene dosage in immortalized cells. Prohibitin mRNA is expressed to a greater extent in all immortalized cell lines; and the elevated expression is almost exclusively the result of an increase in the 1.9 kb transcript. The increased transcription is reflected in elevated protein levels. Western analysis shows that immortalized cells contain from 50 to 200% more prohibitin protein than normal cells. These results suggest that if prohibitin is involved in cell immortalization it 1) is mutated in immortalized cells and the altered form of the protein does not possess the required antiproliferative activity or 2) is subject to a secondary regulatory mechanism whose alteration results in the repression prohibitin's normal suppressor activity.

- V-1058** Normal Endothelial Cell Proliferation And Control Using A Novel Culture Fluid. V. CALDWELL, V. Mayer, R. Wilkinson, and A.R. Torres. HyClone Laboratories, Inc., 1725 S. HyClone Road, Logan, UT 84321.

Gene therapy involves the introduction of cells containing recombinant genes into patients to correct genetic defects. Cultured endothelial cells are commonly used for this new type of therapy as they grow in the vasculature. Improved culture methods would be useful as primary endothelial cells develop abnormal morphology and senescence after several passages when cultivated in standard 10% FBS. We describe a new plasma-based cell culture fluid which allows normal primary bovine aortic endothelial cells to be cultured for extended periods of time with normal morphology and Factor VIII expression. The addition of simple biochemical supplements induce the cultures to enter log-phase growth. These cultures can be withdrawn from log-phase growth by removing the supplements. Normal morphology and Factor VIII expression are maintained in and out of log phase with this culture system. The ability to take the cells in and out of log-phase growth has potential for improving transfection rates with recombi-

nant retrovirus and for maintaining normal cells in culture for long periods of time.

**V-1059** E1A-NR.1: An E1A-Immortalized Retinal Cell Culture With Serum-Sensitive Glial/Neuronal Properties. G.M. SEIGEL, F.Q. Liang, and M. del Cerro. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

The development of the highly complex and laminated neural retina is an orchestrated series of events led by both genetic and environmental factors. Tissue culture studies have helped identify many of these factors, but progress has been hampered by the phenomenon of primary cell senescence and the inability to obtain large numbers of particular cell types. To address these issues, an immortalized retinal cell culture was established by infection of E17 rat retinal tissue with a replication-incompetent retrovirus carrying the 12S E1A immortalizing gene. The resulting mixed neuroretinal cell culture was designated E1A-NR.1 and has been maintained beyond 18 passages *in vitro* with continuing expression of the E1A immortalizing gene product. Under culture conditions containing 10% fetal calf serum, E1A-NR.1 exhibited a primarily glial cell phenotype, as evidenced by flat morphology, >99% immunoreactivity to the glial markers S-100 and glial fibrillary acidic protein, as well as immunonegativity to the neuroendocrine marker PGP 9.5. Following two days in Neumann & Tytell's serumless medium, morphologically neuronal-like cells appeared, while immunoreactivity to PGP 9.5 increased markedly. At the same time, immunoreactivity to S-100, GFAP, and the E1A gene product decreased to barely detectable levels. These results show the potential usefulness of E1A-NR.1 cells in retinal cell growth and differentiation studies, as phenotype and E1A expression may be manipulated by changing culture conditions. More definitive conclusions can be drawn once clonal populations derived from E1A-NR.1 are analyzed and specific cell types are identified under varying culture conditions.

**V-1060** Primary Culture Of Carp, *Cyprinus carpio*, Hepatocytes. R. Böhm<sup>1</sup> and H. Segner<sup>2</sup>. <sup>1</sup>Department of Zoology, University of Karlsruhe, D-76128 Karlsruhe, FRG; <sup>2</sup>Department of Chemical Ecotoxicology, Centre for Environmental Research, D-04318 Leipzig, FRG.

The primary culture of teleost hepatocytes is a comparatively unexplored field, despite its great potential for physiological and ecotoxicological research. Hepatocytes from carp liver were prepared by collagenase perfusion and were cultured as monolayer in serum- and hormone-free media at 20° C. Contrary to what has been observed for hepatocytes of other fish species, carp liver cells readily attached to plastic culture plates. Viability – as judged from the retention of intracellular enzymes and from the cellular energy charge – remained high during a 72-hour incubation period. Carp liver cells in culture did not flatten as it occurs with rat hepatocytes. Within the

first 12 hours of incubation, the initially single cells started to reaggregate and to form junctional complexes. Bile canaliculi, however, did not develop, probably because the *in vivo* formation of bile capillaries in carp liver requires cooperation both of parenchymal cells and of bile ductular cells, the latter being absent in the *in vitro* system. Cultured carp liver cells conserved many aspects of liver-specific metabolism, e.g. maintenance of the activity levels of key metabolic enzymes, high rates of protein and fatty acid synthesis and secretion of lipoproteins. Moreover, the hepatocytes maintained remarkably high concentrations of glycogen (>1000 mg glucosyl units/g protein). Even when incubated in nutrient-free salt solutions, only minor decreases of cellular glycogen levels occurred. The stability of glycogen is a peculiar feature of isolated carp liver cells hitherto reported neither for mammalian nor for piscine hepatocyte cultures.

**V-1061** Mycoplasma Testing Of Cell Cultures By A Combination Of Direct Culture And DNA-Fluorochrome Staining. D.J. Lundin and C.K. Lincoln. Bionique Testing Laboratories, Inc., Saranac Lake, NY 12983.

The laboratory evaluated the most recent 1,000 submissions for mycoplasma testing by a multimedia direct culture and DNA-fluorochrome staining assay. The direct culture procedure included 3 different media formulations (Complete Fortified Commercial, Modified Hayflick, and Heart Infusion), broth and agar. Plates were incubated aerobically and anaerobically. Direct cultures remained on test for a total of 28 days. The DNA-fluorochrome staining was performed after a 96-hour co-incubation with an indicator cell line (VERO ATCC #CCL81).

102 samples were determined to be positive for mycoplasma contamination or 10.2% of the total 1,000 submissions. 81 samples or 79.4% of the positive cultures were detected by both direct culture and DNA-fluorochrome staining. 15 samples or 14.7% of the positive cultures were detected by direct culture only, the DNA-fluorochrome staining yielded false negative results. 6 samples or 5.9% of the positive cultures were identified only by DNA-fluorochrome staining. Direct culture yielded 0.6% false negative results. In our experience the combined multimedia direct culture and DNA-fluorochrome staining assay is the most accurate mycoplasma testing procedure. These results show that there is a significant level (10.2%) of mycoplasma contamination of continuous cell lines that are routinely tested. We project a greater contamination rate in cell cultures that are not routinely tested for mycoplasma.

**V-1062** Antibiotic Resistance Of Mycoplasma Isolates From Cell Cultures. C.K. Lincoln and D.J. Lundin. Bionique Testing Laboratories, Inc., Saranac Lake, NY 12983.

This laboratory evaluated its most recent antimicrobial susceptibility test results to document resistance to antibiotics considered to be antimycoplasma agents. Discs were applied to the surface of Commercial Forti-

fied agar plates incubated with mycoplasmal isolates from cell culture samples submitted for mycoplasmal elimination. Except for tylosin, BBL Sensi-discs® were used and positioned according to standard procedure. Results were interpreted qualitatively on a basis of presence or absence of a definite zone of inhibition. Mycoplasmal species identified among the isolates tested included 13 *M. hyorhinis*, 9 *M. arginini*, 8 *M. orale*, 6 *M. fermentans*, 3 *M. salivarium*, 1 *M. bovoculi*, 1 *A. laidlawii*, and 6 unknown species. The routine use of antibiotics in cell cultures may select for resistant strains; the blind use of any antibiotic agents for elimination purposes may well be ineffectual.

Antibiotics	Conc.	#Tested	#Resistant	%Resistant
Chloramphenicol	30 mcg	47	14	30
Chlortetracycline	30 mcg	46	5	11
Ciprofloxacin	5 mcg	34	5	15
Erythromycin	15 mcg	46	45	98
Gentamicin	10 mcg	46	37	80
Kanamycin	30 mcg	45	33	73
Lincomycin	2 mcg	46	13	28
Neomycin	30 mcg	36	31	86
Nystatin	100 mcg	35	33	94
Spectinomycin	100 mcg	36	5	14
Streptomycin	10 mcg	42	37	88
Tetracycline	30 mcg	44	6	14
Tylosin	100 mcg	38	8	21

**V-1063** Jumbocytes In Mitosis, A Putative Source Of Genetic Heterogeneity. J. Leighton. Aeron Biotechnology, San Leandro, CA 94577.

On examination of long-term cultures of cells derived from normal tissue, attention is directed to the predominant cell types. When cytologic events of transformation are seen in some living cultures, rare very large cells with bizarre nuclei are often ignored. These very large cells, found commonly during the "crisis" phase, may be cytologic movers in the transformation to immortal cells, because one finds such cells with four or more bizarre metaphase plates. Occasional viable daughter cells from such mitoses may have new qualities including immortality. *Jumbocyte* may be a better name than *giant cell* to designate this fecund cell. Data on the transformation of normal human and rodent cells with the coincident presence of jumbocytes have been published by many (J. Exp. Med., 97:525, 1953; Science, 123:502, 1956; Proc. Soc. Exp. Biol. & Med., 92:867, 1956; Can. Res., 17:668, 1957; N.Y. Acad. Special Pubn., 5:362, 1957; Japan J. Exp. Med., 35:513, 1965). I have observed that some established cell lines generate jumbocytes in large numbers in planar culture as a response to gradients of pO<sub>2</sub> and of temperature (NBT-II), and on exposure to substances such as ouabain (MDCK) or to methyl nitrosourea (RT4). The fecund jumbocyte may be a cytologic form by which low grade tumors become more malignant.

**V-1064** Cells On Fibers (Rotated and Corrugated). ROBERT CLYDE, Clyde Engineering, P.O.B. 740644, New Orleans, LA 70174.

Fungi and some mammalian cells (especially liver cells) need oxygen. An efficient way to supply oxygen is provided when corrugated fibers are rotated in a half full rotary biological contactor as described in newly issued patent 5,256,570. Liquid is carried up into the vapor space and falls down through holes in the valleys. Mass transfer to drops is much higher than to a flat surface.

In another design, fiber discs are rotated in a vertical reactor. Nutrient comes down from the top and makes a large circle before falling through holes to the next disc, thus contacting more cells than when put zig zag through small stationary discs. Plant, insect and bone marrow cells also grow on fibers. A reactor with a strong, rare earth magnet agitator is being built.

**V-1065** Inhibitory Effect Of Albenz On Succinate Dehydrogenase Of Tape Worm (Neokrimia Singhia). M.R. SIVA SAI KUMARI And Ratnamala Rao. Department of Zoology, Osmania University, Hyderabad-7 (AP) India.

The succinate dehydrogenase (SDH) is an essential enzyme of Tricarboxylic acid (TCA) cycle. The high activity of SDH in this Cestode parasite was indicative of the operation of CO<sub>2</sub> fixation pathway (partial reverse of TCA cycle). It is estimated biochemically in the control and treated parasites. Under drug stress, the depletion of SDH activity was observed. It indicates that the decreased operation of Krebs' cycle probably due to limiting the flow of substrates into the cycle and depressed rate of metabolism.

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  1. Pipettes, serological
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    - b. 1-ml, Pyrex, no. 7086, Corning<sup>5</sup>

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